# REPORT 56

[redacted]

SWEEP

SOIL AND WATER

ENVIRONMENTAL

ENHANCEMENT PROGRAM

PAMPA

PROGRAMME D'AMELIORATION

DU MILIEU PEDOLOGIQUE

ET AQUATIQUE

Canada

Ontario

[redacted]

SWEEP

*is a $30 million federal-provincial agreement, announced May 8, 1986, designed to improve soil and water quality in southwestern Ontario over the next five years.*

## PURPOSES

*There are two interrelated purposes to the program; first, to reduce phosphorus loadings in the Lake Erie basin from cropland run-off; and second, to improve the productivity of southwestern Ontario agriculture by reducing or arresting soil erosion that contributes to water pollution.*

## BACKGROUND

*The Canada-U.S. Great Lakes Water Quality Agreement called for phosphorus reductions in the Lake Erie basin of 2000 tonnes per year. SWEEP is part of the Canadian agreement, calling for reductions of 300 tonnes per year — 200 from croplands and 100 from industrial and municipal sources.*

PAMPA

*est une entente fédérale-provinciale de 30 millions de dollars, annoncée le 8 mai 1986, et destinée à améliorer la qualité du sol et de l'eau dans le Sud-ouest de l'Ontario.*

## SES BUTS

*Les deux buts de PAMPA sont: en premier lieu de réduire de 200 tonnes par an d'ici 1990 le déversement dans le lac Erie de phosphore provenant des terres agricoles, et de maintenir ou d'accroître la productivité agricole du Sud-ouest de l'Ontario, en réduisant ou en empêchant l'érosion et la dégradation du sol.*

## SES GRANDES LIGNES

*L'entente entre le Canada et les États-Unis sur la qualité de l'eau des Grands Lacs prévoyait de réduire de 2 000 tonnes par an la pollution due au phosphore dans le bassin du lac Erie. PAMPA fait partie de cette entente qui réduira cette pollution de 300 tonnes par an — 200 tonnes provenant des terres agricoles et 100 tonnes provenant de sources industrielles et municipales.*

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TECHNOLOGY EVALUATION AND DEVELOPMENT SUB-PROGRAM

# YIELD REDUCTION EFFECTS OF CROP RESIDUES IN CONSERVATION TILLAGE

FINAL REPORT

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## EXECUTIVE SUMMARY

### FIELD STUDIES

The presence of crop residues on the soil surface in conservation tillage systems affected soil water content and temperature,and plant growth. When the spring season was dry, residues retarded evaporation resulting in soil temperatures cooler in comparison to a conventional tillage system. However, the extra water retained in the soil also promoted plant growth. During a wet season, plants appeared pale and growth was stunted due to cooler soil temperatures and wetter soils. Nevertheless, soil temperature and moisture measurements could not totally explain the reduction observed in corn plant development in the presence of specific plant residues, particularly residues of red clover and canola.

Phenolic acids (PCs) and volatile fatty acids (VFAs) generally were not found in concentrations considered to be phytotoxic in field soil samples. Field sampling in the spring showed the presence of PCs which tended to decrease in concentration from May to June. However, within 48 hours after a 40 mm rainfall event in late June, PCs were detected in plots with residue cover. The presence of VFAs was detected only once during the three years of study in the field. This was after a shower in early June from a plot receiving rye residues (4 t $ha^{-1}$).

The addition of calcium nitrate to soil amended with crop residues significantly reduced accumulation of VFAs in a

laboratory study. However, under field conditions the addition of calcium nitrate with the seed did not overcome the yield reduction effects of rye residues.

**LABORATORY STUDIES**

The production of VFAs is dependant on the nature of the crop residue, soil water content and temperature. Green, readily decomposable residues such as legumes had the greatest potential for the accumulation of VFAs when soil temperatures were high (15-25°C) and soil water potential was above field capacity. The potential for VFA production decreased as the plant materials matured to harvest stage and underwent weathering.

There was little difference in the accumulation of VFAs under soil moisture conditions ranging from field capacity to saturation. Phytotoxic concentrations could be produced in the laboratory within 48 hours of incubation, a period which could be expected in the field.

The accumulation of VFAs was much greater at high temperatures (25°C) than at low temperatures (4°C). During incubations there was a lag period of about 2 days followed by a rapid increase in VFA accumulation to a maximum at about 14 days. This was followed by a rapid decrease in soil VFA concentration indicating that these compounds were readily utilized by soil microorganisms. A second but much smaller peak was observed on day 28, especially for rye and soybean residues. Under low temperatures (4°C), VFA accumulations were

small but steady over the entire incubation period. Total phenolic concentrations were never as high as those found for VFAs. This may be because VFAs are a by-product of anaerobic decomposition whereas PCs are released as the residue undergoes decomposition.

Bioassays using corn and winter wheat were performed to assess phytotoxic affects. Selected VFAs and PCs inhibited germination and early growth of corn, although not all hybrids were affected equally. Phytotoxicity increased with decreasing solution pH. Combinations of the phenolic acids appeared to affect corn radicle growth in a non-additive manner.

The winter wheat bioassay showed that VFAs were more inhibitory to plant growth than PCs. Acetic acid was inhibitory to both radicle and coleoptile growth, irrespective of crop variety or VFA concentration tested. Propionic and butyric acid also inhibited plant growth but to a lesser extent than acetic acid. Vanillic acid was inhibitory to germination and early seedling growth whereas *p*-hydroxybenzoic acid and *p*-coumaric acid stimulated early seedling growth.

In conclusion, the field research indicates that phytotoxins are produced from decomposing crop residues which can significantly reduce plant growth. However, the nature of these phytotoxic compounds is not clear. VFAs and PCs accumulated in the soil to phytotoxic concentrations during the decomposition of particular crop residues in laboratory

studies. However, these compounds were not detected in field studies. Future research should consider the dynamics of both the production and utilization of phytotoxin compounds during decomposition of crop residues. In addition, there is a need to examine other potential phytotoxins, such as the volatile $C_6$ through $C_9$ organic compounds.

# 1.0. CHAPTER 1 GENERAL INTRODUCTION

In 1983, 38% of U.S. cropland was in some form of conservation tillage and it is estimated that by the year 2000, 65% will be managed this way. Conservation tillage is defined as a tillage system which reduces the loss of soil and water and is usually associated with higher surface crop residue levels, especially in zero-tillage systems (Mannering and Fenster, 1983).

In southern Ontario the acceptance of zero-tillage systems for corn production has been slow because research on long term plots with medium- and fine textured soils indicates an average 10% yield reduction compared to conventional use of the mouldboard plough in the fall (Vyn et al., 1983). Corn yield reductions with zero tillage, relative to conventional tillage, have generally been most severe when corn follows corn; yield reductions have been insignificant when corn follows sod crops (Vyn et al., 1979) or soybeans (Vyn, 1987). However, occasionally severe yield depressions (i.e. greater than 15%) have occurred when corn has been zero-till planted after spring-killed rye cover crops (Raimbault et al., 1990) and spring-killed red clover and alfalfa (Vyn, 1987). Plant growth inhibition by increased percentage of surface residues, due to conservation tillage practices, has been suspected in several of the major cropping sequences in Ontario.

Phytotoxicity or allelopathy is the harmful effect on the growth of one plant species by the same or another species

through the production of toxic substances. These toxic compounds may be leached from crop residues or produced by saprophytic microorganisms during residue decomposition under anaerobic conditions (Cochran et al., 1977). The phytotoxic compounds isolated from fresh and decomposing crop residues and from soils belong to several chemical classes including organic acids, lactones, ketones, simple phenols, alkaloids, terpenoids, tannins and others (Elliot et al., 1978, Rice, 1984). The major products of anaerobic fermentation are volatile fatty acids, primarily acetic, propionic and butyric, with reports of small amounts of formic, lactic and valeric acids also present. The water-soluble components and the readily decomposable cellulose and hemicellulose in the residues appear to be critical substrates in anaerobic fermentation (Lynch, 1983).

Presumably for a compound to affect crop yields, it must be either water soluble and diffusing through the soil from the site of production to the seedling, or have a vapour pressure high enough to diffuse to the plant. A major factor contributing to yield reduction with conservation tillage may be production of phytotoxins during the flush of decomposition that coincides with the early stages of crop growth.

The phytotoxic effects of crop residues under conservation tillage have not been investigated in Ontario but there is indirect evidence of their involvement. Previous research at the University of Guelph and on-farm conservation

field trials in Ontario showed that moving the crop residues from the planting row increased corn yields (but not always significantly) compared to not moving the residue under zero-tillage (van Roestal, 1983). Further, the yield reduction associated with zero-tillage compared to conventional tillage when corn followed corn was not observed during the two year study providing residues were removed.

More recent research on residue movement by mechanical means (planter-mounted 'trash whippers') has shown that removal of surface residue out of the row area was often associated with faster rates of early corn growth and earlier flowering when corn was zero-till planted after corn and barley (Vyn, 1987). Corn yield increases due to residue removal from the row area were significant on 2 of the 3 soil types investigated during the three year study. Mechanical movement of surface residue from the row area did not increase early corn growth or the final grain yield in situations where corn was zero-till planted after crops such as soybeans, red clover, or alfalfa (Vyn, 1987). A reported field study on surface residue placement with corn in the United States (Cruse, 1987) concluded that residue-free bands of 15 or 33 cm widths resulted in corn yields which were similar to those with zero-till planting on a completely bare soil. In the latter study, there was no indication of what the relative effects of various crop residues might be.

Another case of indirect evidence of the phytotoxic

effects of crop residues under conservation tillage was observed in Ontario research on corn response following winter cover crops of rye and wheat (Raimbault et al., 1990). Rates of corn growth and development and final dry matter yield were depressed whenever rye or wheat was grown as a winter cover crop (at two locations over a three year period). A significant reduction (average of 20%) in corn yield was observed where corn was zero-till planted into rye stubble; spring primary tillage prior to planting corn tended to partially overcome the negative effects of rye on corn (average yield reduction of 10% relative to corn planted after no cover crop). Removal of above-ground rye residue versus chemically killing rye the day before planting corn had no influence on the subsequent corn yields. The data suggest that the detrimental influence rye exerts on the corn crop was due to soil or root related phytotoxins. Much of the detrimental impacts of rye on corn were avoided by killing the rye (either chemically or mechanically) at least two weeks prior to planting corn. Early chemical kill appeared to be particularly important when corn was zero-till planted into the rye stubble.

The following report is a summary of the field and laboratory experiments conducted under contract number 01686-7-0339/01-SE for the three year period from 1988 to 1990.

Throughout this study similar methodologies were used to determine the presence of phytotoxins. Bulk composites of soil

were taken from experimental plots, placed on ice in coolers and immediately transported to the laboratory. Soil extracts were obtained by taking 50 g of fresh soil, adding 50 mL of refrigerated, distilled, deionized water. The soil-water mixture was centrifuged at 6451 x g for 15 min (10°C). The supernatant was filtered through Whatman #4 filter paper for phenolic acid analysis. An aliquot was further filtered through a 0.45 μm pore diameter cellulose millipore filter prior to volatile fatty acid (VFA) analysis.

### 1.1. VFA ANALYSIS

Purified, concentrated formic acid (Aristar, BDH Chemicals) was added to the filtered soil extract (50 μL of formic acid to 450 μL of extract) and 1 μL of this solution was injected directly into the gas chromatograph (GC - Varian 3300). The GC was equipped with a glass column (91 cm X 4 mm internal diameter) using the same column packing and equipment settings as Paul and Beauchamp (1989). Standard solutions were made with combination of different concentrations of acetic, propionic and butyric acid.

### 1.2. TOTAL PHENOLIC ACID ANALYSIS

Measurements of total phenolic compounds (PCs) in the soil extract were made by adapting the methodology of Swain and Hillis (1959) for use on an autoanalyzer system (L.J. Evans and D.A. Tel, personal communication). The method used Folin-Denis reagent, EDTA (sodium tetraethylene diamine tetraacetate) and a diluent (Brij 35); absorption was measured

using a 15-mm flow cell set at 720 nm. Standard solutions were made with salicylic acid.

## 2.0. CHAPTER 2 CORN/RYE FIELD EXPERIMENTS - 1988, 1989

### 2.1. OBJECTIVE

To evaluate surface placement of coarsely-chopped overwintered corn and fresh rye plant residues for phytotoxin production using corn as the test crop.

### 2.2. METHOD AND MATERIALS

#### 2.2.1 Experimental Design

The plot design consisted of four repetitions of six treatments in plots 3m wide by 15m long. A 1m buffer was left at the end of the plot before the subplots were staked out to incorporate the manually irrigated and garden blanket treatments. The 3m width provided room for 4 rows of corn. The inner two rows of each plot were the rows used for sampling of plant material and final harvest.

Corn was planted conventionally on May 18th, 1988 on plots, previously cropped to soybeans, which had been tilled. At time of planting the inner two rows were planted at 120 000 plants•ha$^{-1}$ but after the second plant sampling the stand population was reduced to 60 000 plants•ha$^{-1}$. Fertilizer was broadcasted and incorporated prior to seeding at rates of 104 kg•ha$^{-1}$ urea and 60 kg•ha$^{-1}$ $K_2O$. At time of planting an additional 20 kg•ha$^{-1}$ of $K_2O$ and $P_2O_5$ was side-dressed.

Residues were placed between the seed rows within 48 hours of planting. A 5 cm strip over the seed row was left bare. Residues were applied at 6 and 12 t•ha$^{-1}$ dry chopped (≤

5 cm) corn and 2 and 4 $t \cdot ha^{-1}$ fresh green rye (harvested by a flail carter). The control treatment was left bare. An inert material ($2m^2$) referred to as garden blankets (Dupont), were used to simulate the physical effects of the residue cover. All treatments had $2m^2$ subplots which were irrigated with 25 L of water, evenly distributed, 3 times weekly to maintain moist conditions.

Soil samples were taken weekly beginning May 25th and continued June 2nd, 9th, 16th, 23rd and 30th for analysis of VFAs, PCs and gravimetric water content. Soil samples were taken between the row crop and through the residue approximately 20 hours after the second application of water for the week. The soil composite consisted of 10 soil cores to 5cm depth for the large plots and 5 soil cores for the subplots. Thermocouples were placed at a 5cm depth below soil surface to measure soil temperatures. Temperature highs, lows and averages were recorded for a 24 hour period during early crop growth. Plant samples were taken for dry matter on June 14th and July 7th.

### 2.2.2 Weather

Climatic data for the research site is summarized by month in Table 2.1. The monthly total and mean values portrays an early growing season where temperatures were above normal and rainfall below normal. This trend continued into June and resulted in the crop being subjected to drought stress. Drought stress was reduced by irrigation and natural rainfall

in the second week of July. Overall the growing season had above-normal temperatures and below normal rainfall.

**2.3. RESULTS - 1988**

The first bulk soil samples were taken May 25th before emergence but analyses revealed no VFAs or PCs. There were no propionic or butyric acids and only small amounts of acetic acid or total PCs measured in soil sampled over the early growing period. Figure 2.1 and 2.2 show acetic-C and PC-C for the 4 $t \cdot ha^{-1}$ rye treatment found on their respective sampling dates. It is interesting to note a slight accumulation of VFAs on the June 16th sampling on the nonirrigated treatment. A rain shower (4.4 mm) had occurred the previous night which may have leached phytotoxins from the residues or provided conditions for there production. The irrigated treatment had only a very slight increase in total VFAs. There was also a flush of total PCs for the same date and treatment. There was only the presence of acetic acid detected by the GC. All other treatments had very low amounts of acetic acid on any given sampling date and is at the lower limits of detection. This was also the scenario with the detection of PC-C.

**2.3.1 Plant Weight and Temperature**

Table 2.2 shows the mean plant weight (mg) from the June 14th sampling as well as the temperature recorded for several days on each treatment. The effect of water and residues is significant in reducing plant weight in the early growth period. The greatest plant weight was recorded under the bare

ground control treatment with the garden blanket as a subtreatment. Whereas, the lowest plant weight was recorded by the corn residue at both heavy and light application with the irrigation subtreatment. The control with garden blanket was significantly different from all other treatments. The control with garden blanket and irrigation is significantly different from all treatments where irrigation was part of the management system. There is no significant difference between the heavy/light application of either the rye or corn residues when there was no irrigation. There was significant difference between the corn residue applications with the irrigation treatment included. It is interesting to note that all irrigated treatments under crop residues resulted in lower plant weights in comparison to the treatments without irrigation. The second sampling (July 11[a]) resulted in the control with garden blanket and garden blanket with irrigation having the greatest plant weight; 759 and 750 mg respectively. These treatments were followed by heavy corn (563 mg), light corn (528 mg), control (525 mg), heavy rye (504 mg), light rye (498 mg), irrigated control (453 mg), irrigated heavy rye (405 mg), irrigated heavy corn (326 mg), irrigated light corn (323 mg) and irrigated light rye (321 mg). There are no significant differences between treatments of similar management except between the control and control with garden blanket and garden blanket with irrigation.

The temperatures listed by date and treatment for a

specific time period reveals the cooler temperatures, of the soil 5 cm below the soil surface under the residue mat, under the heavy application of natural residues. The application of water further reduced the temperatures recorded. The bare ground control plot temperatures were higher in comparison to the corn and rye residue covered plots. The addition of the garden blanket to the control plot resulted in a temperature increase when comparing temperatures recorded on the bare ground control. The addition of water to the garden blanket subtreatment resulted in slightly cooler temperatures in comparison to garden blanket subtreatment. Figures 2.3 and 2.4 gives examples of temperature high and lows recorded over a 24 hour period (June 14$^{th}$-June 15$^{th}$) for the rye treatments.

**2.3.2 Grain Yield**

The final grain yield (Table 2.3) represented at 15.5 % moisture revealed some interesting results. The greatest yield was obtained from the heavy application of rye (4 $t \cdot ha^{-1}$) under the early irrigation regime (8.43 $t \cdot ha^{-1}$) while the lowest yield (4.79 $t \cdot ha^{-1}$), almost a 50 % reduction, was the treatment consisting of the heavy rate of corn (12 $t \cdot ha^{-1}$) also with the irrigation during germination and early seedling growth. There was no significant difference between like treatments with regard to residue type and rate of application The only significant difference occurred between the heavy rye application with irrigation and the heavy rye application comparison. There were also significant differences between

the control with irrigation treatment and the heavy rye, heavy corn and light corn residue application with the early irrigation subtreatment. The irrigated garden blanket treatment final yield was significantly different from the light and heavy rye residue application with irrigation. The control with garden blanket (simulating presence of residue without possibility of leaching compounds) was not significantly different from the light or heavy residue application of either the corn or rye residue. The same can only be said with regard to the corn residue application with irrigation when comparing to the control with garden blanket and irrigation. Overall yield trends resulted in the corn treatments having a yield less than any of the control treatments. The rye treatments had a completely opposite effect on the final yield. Rye applied at the heavy rate enhanced the final yield even though this was the only treatment with measurable VFAs in the early growing season.

The rye residue was rapidly decomposed during the growing season no matter what rate of application. The corn residue, however, appeared to be relatively undecomposed when harvest took place at the end of the field season. The persistence of corn residues may result in the slow release of toxins over time. Guenzi et al. (1967) found that after 8 weeks of exposure to field conditions, there were essentially no water soluble toxic components for wheat and oat residues whereas, corn had water soluble components after 22 - 28 weeks of

exposure. Rice (1984) had similar results and Kimber (1973) found that after 18 days rotting straw had no affect on germination of wheat.

**2.4. RESULTS - 1989**

During the 1989 growing season, rye and corn residues were evaluated, for the second time, for phytotoxin production using corn as the test crop. Crop residues were placed between the seed rows. Fibreglass blankets (DuPont) were used, to simulate the physical effects of residues, ie. soil warming, without the contribution of phytotoxic compounds, as a control. There were also bare ground control plots. Subplots received 25 L of water 3 times a week if it had not rained a significant amount the night before or the day of application. Bulk soil samples were taken between the row crop and through the residue approximately 20 hours after the second application of water for the week, for the first four weeks during the early growing period. During the 1989 season, high, lows and average temperatures were recorded hourly, 7 days a week for the first 8 weeks and every 6 hours for the remainder of the season.

Water extracts were prepared from the bulk soil samples (soil:water, 1:1) and analyzed for VFAs and total PCs. Gravimetric water contents were also obtained at time of sampling. Other measurements included plant weights sampled on June 21[a] and July 14[b], plant height July 25[b] and the final grain yields calculated at 15.5 % moisture for each treatment.

The corn was planted under good moisture conditions on May 19th at 120 000 plants•ha$^{-1}$ and later thinned to 60 000 plants•ha$^{-1}$. The plots were placed on an area previously cropped to soybean, fall ploughed, and fertilizer incorporated (317 kg•ha$^{-1}$ $NH_4NO_3$) before planting. At time of planting 0-20-20 was side dressed at 200 kg•ha$^{-1}$ to have an uniform fertility trial. The fresh, green rye was placed immediately after planting and the chopped corn residue was placed two days following planting. The average rates of residues placed on the plots were rye 2 and 4 t•ha$^{-1}$ and corn at 6.5 and 13.2 t•ha$^{-1}$. Composite soil samples were taken on June 1st, 5th, 8th, 15th, 23rd and 29th.

### 2.4.1 Weather

Table 2.4 summarizes total, mean and average values by month for temperature high, temperature low, rainfall and corn heat units for the 1989 growing season. The early growing season temperatures were around the norm but the precipitation was above average for the months of May and June. After a wet spring, the month of July was droughty. The comparison of early growth seasons between the two crop years, keeping all other management practices similar, results in an overall dry year and wet year for the two seasons.

### 2.4.2 Soil Extracts

There were no significant quantities of measurable VFAs in soil sampled measured over the early growing period although VFAs maybe present at the border of the

instrumentation sensitivity. The same results were obtained for PCs even though overall treatments average soil moisture content on any given sampling date was greater than 25 % by weight.

### 2.4.3 Visual Observations

Growth throughout the season varied by treatment. In general plots with residue had plants which were smaller, paler in colour and showed signs of phosphorus deficiency within the first 5 weeks of emergence. In July, during the drought period, these same treatments continued to grow and did not show any drought stress in comparison to the bare ground control plots. Table 2.5 summarizes the mean plant weight (mg) from the two samplings of plant material for each treatment plus the plant height measured July 25[a]. The effect of water and residues reduced plant weight in the early growth period but in most comparisons, there is no significant difference. The exception is the light application of rye and the irrigation treatment of the light rye application. The 1989 growing season resulted in a central position for the control treatment with garden blanket and irrigation and was significantly different from the control, light rye and control with garden blanket treatments. By the second sampling there were no significant differences between treatments of similar residues even when irrigation subtreatments are included. The light, irrigated rye treatment had the largest mean plant weight and the heavy, irrigated corn treatment the

lowest mean plant weight. It is interesting to note that by this time in the growing season that the irrigated subplots of light rye, control and heavy rye were the top three in mean plant weight surpassing respective field condition plots. By July 25th, the height parameter had evened out with no significant difference between the plots amended with residue. The only significant difference was between bare ground control and bare ground control with irrigation.

### 2.4.4 Grain Yield

Final grain yields were taken at the end of October (Table 2.6). The highest yield was recorded for the heavy rye under the irrigation subtreatment followed by the heavy rye and light irrigated corn treatments. The three lowest yields were obtained from the control plot with irrigation, control with garden blanket and irrigation and the lowest was control with garden blanket. None of the control treatments were significantly different from each other nor were treatments of the same residue tonnage. Significant differences occurred when comparing natural residue to simulated residue response. The control treatments with garden blanket and with or without irrigation were significantly different from all rye treatments and irrigated corn treatments. The control with irrigation is significantly different from irrigated, light corn, heavy rye and irrigated, heavy rye.

## 2.5. SUMMARY

### 2.5.1 VFAs and PCs

The presence of VFAs or PCs was detected only once in the two year study after a small precipitation event. The amounts found were at the lower limits of the instrumentation sensitivity. The fact that VFAs and PCs were detected indicates the possibility of these chemical groups as phytotoxic to germination and early seedling growth.

### 2.5.2 Yield

The two growing seasons were quite different in 1988 and 1989 resulting in the final yield differences. The 1988 season was dry with above average temperatures in comparison to the 1989 season which was wet with average temperatures. The use of the garden blanket to simulate presence of residue, without adding the leaching of compounds aspect of residue, resulted in higher temperatures being recorded. These higher temperatures resulted in enhanced early growth of the test crop. The affect of the crop residues resulted in cooler temperatures which resulted in smaller, paler plants in the early stages of growth. By mid-season these differences in plant weight and height were indistinguishable. The presence of residues, especially the heavy application, benefitted the test crop during the drought periods in the growing season. Drought stress was more extensive in bare ground control plots. Over the two seasons the drought stress was greater in 1988. The grain yield from the 1988 field season showed that

the amount of corn residue had a reducing effect in yield in both irrigated and nonirrigated plots. The 6 $t \cdot ha^{-1}$ residue treatment with irrigation was slightly more productive and was significant at the 5% level. The corn residue treatments had a lower yield than any of the respective control treatments. The rye treatments had a completely opposite effect on the final yield in the 1988 season. Rye applied at the heavy rate enhanced the final yield even though this was the only treatment with measurable VFAs in the early growing season. The irrigated treatment were significantly different at both light and heavy residue applications in the 1988 growing season. The 1989 season resulted in all rye treatments enhancing the yield. Field condition light and heavy corn residues reduced yield slightly in comparison to the bare ground control whereas the irrigated corn residues increased yield. All other control treatments were at least 331 $kg \cdot ha^{-1}$ below natural residue yields. Therefore, the presence of surface residues under droughty conditions results in a possible yield enhancement in comparison to a conventional system.

Table 2.1 Total, mean and average values for weather variables 1988 - Elora Research Station.

| Month | Variable | Total (1988) | Mean (1988) | Average |
|---|---|---|---|---|
| May | | | | |
| | temperature high | | 19.7 | 17.4 |
| | temperature low | | 7.1 | 5.3 |
| | rainfall | 41.8 | | 77.6 |
| | corn heat units | 297.3 | | 223.0 |
| June | | | | |
| | temperature high | | 24.8 | 23.2 |
| | temperature low | | 9.1 | 11.0 |
| | rainfall | 22.8 | | 86.9 |
| | corn heat units | 537.4 | | 594.0 |
| July | | | | |
| | temperature high | | 28.2 | 25.5 |
| | temperature low | | 14.4 | 12.7 |
| | rainfall | 101.3 | | 73.0 |
| | corn heat units | 768.0 | | 718.0 |

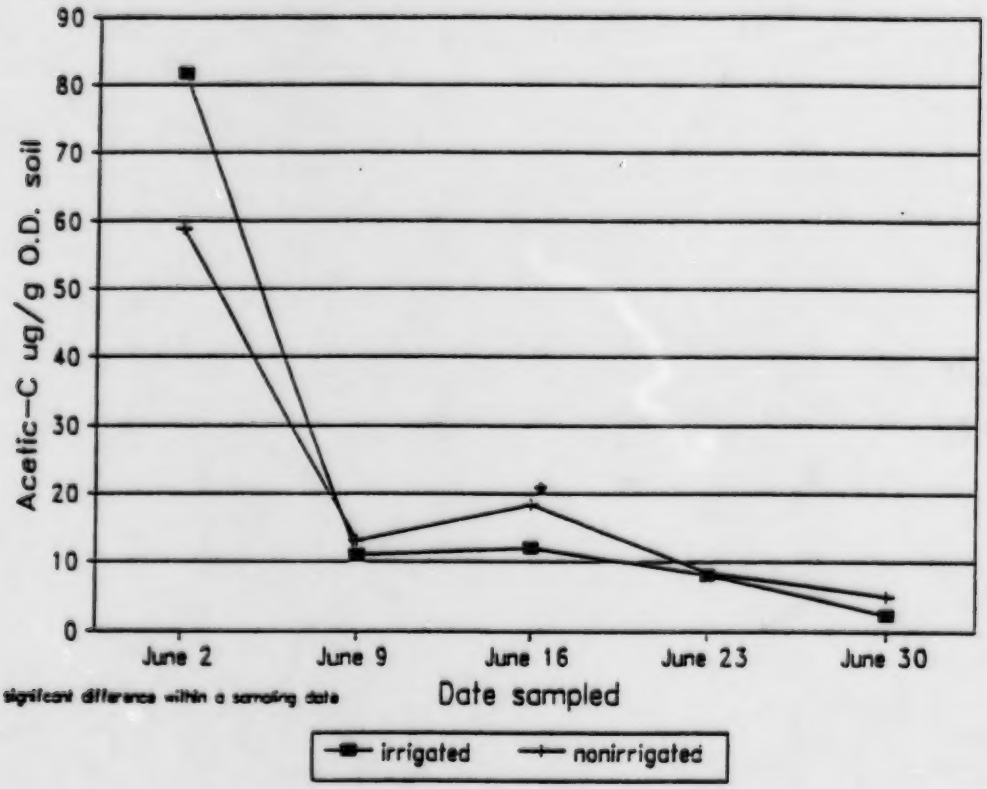
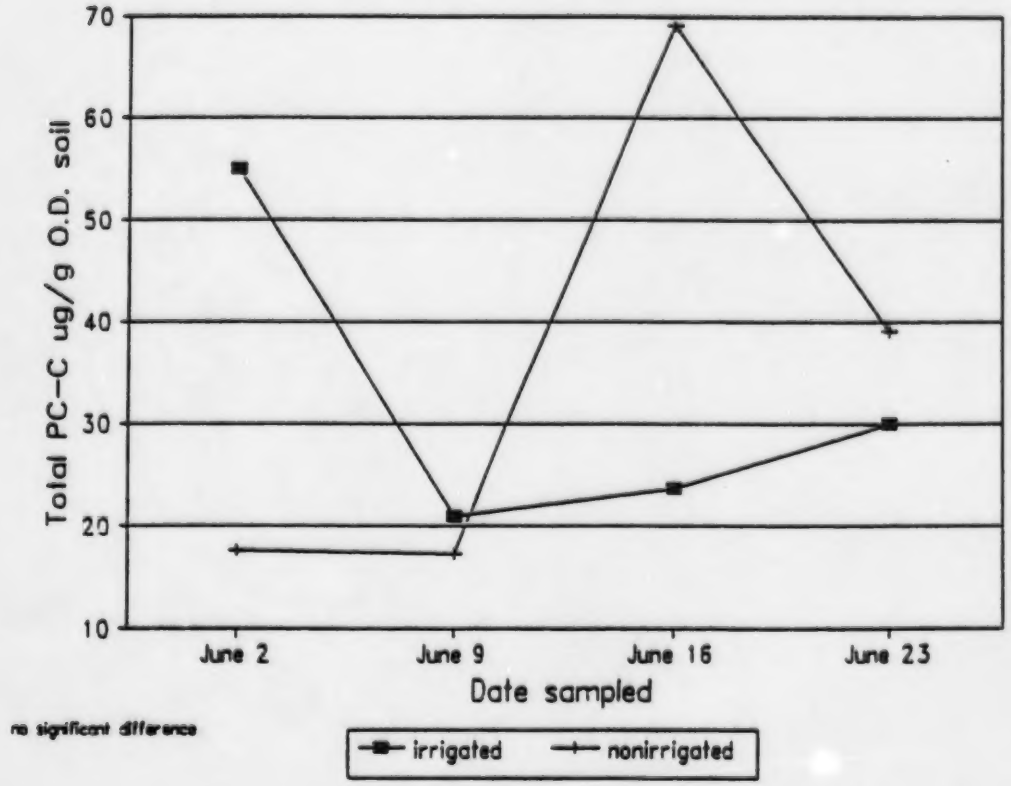



**Figure 2.1 Acetic-C detected from the 4 t ha$^{-1}$ rye treatments by sampling date**




no significant difference

**Figure 2.2 PC-C detected from the 4 t ha$^{-1}$ rye treatments by sampling date**

Table 2.2 Temperatures recorded at 2:00 pm EST from June 7th to July 26th by treatment and average plant weight sampled June 14th.

| Treatment | Plant Weight mg•plant$^{-1}$ | Temperature (2:00 pm EST) (°C) | | | | | |
|---|---|---|---|---|---|---|---|
| | Ju14 | Ju07 | Ju14 | Ju21 | Ju29 | J119 | J126 |
| corn 6t/ha | 278 | 16.65 | 19.04 | 39.27 | 19.58 | 28.41 | 23.33 |
| corn 12t/ha | 260 | 15.68 | 16.55 | 18.88 | 15.32 | 22.38 | 20.15 |
| corn 6t/ha irr.* | 215 | 16.58 | 20.90 | 24.97 | 17.18 | 25.40 | 21.42 |
| corn 12t/ha irr. | 198 | 15.08 | 15.83 | 18.05 | 15.45 | 22.97 | 20.00 |
| rye 2t/ha | 313 | 17.48 | 22.96 | 26.63 | 19.86 | 25.96 | 22.29 |
| rye 4t/ha | 285 | 16.94 | 19.67 | 22.30 | 17.03 | 24.04 | 21.25 |
| rye 2t/ha irr. | 258 | 16.97 | 22.02 | 26.30 | 19.75 | 27.03 | 22.50 |
| rye 4t/ha irr. | 255 | 16.71 | 19.21 | 21.65 | 16.31 | 23.56 | 20.90 |
| control | 303 | 18.20 | 26.62 | 29.49 | 21.84 | 26.5 | 22.60 |
| control irr. | 235 | 17.13 | 28.91 | 30.87 | 23.80 | 26.00 | 21.97 |
| control gb.~ | 548 | 19.79 | 26.78 | 28.73 | 22.71 | 27.48 | 22.69 |
| control gb., irr. | 335 | 19.77 | 24.44 | 27.49 | 18.55 | 25.09 | 21.46 |

* irrigated
~ garden blanket

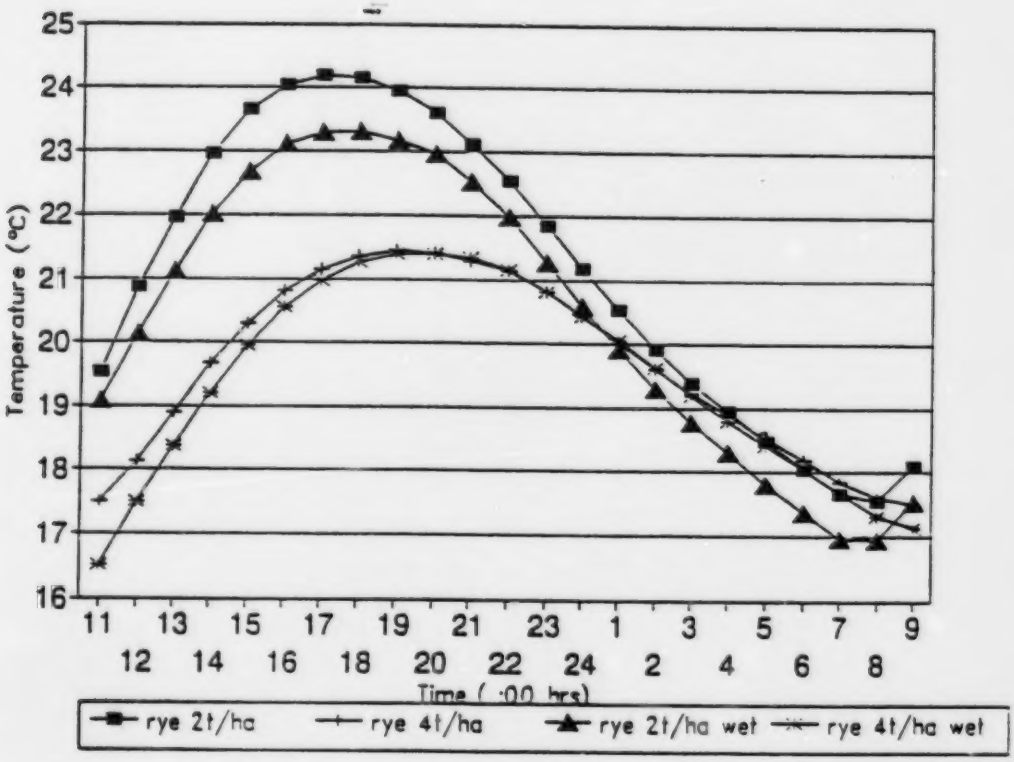



**Figure 2.3 Temperature highs for all rye treatments (June 14-15, 1988)**

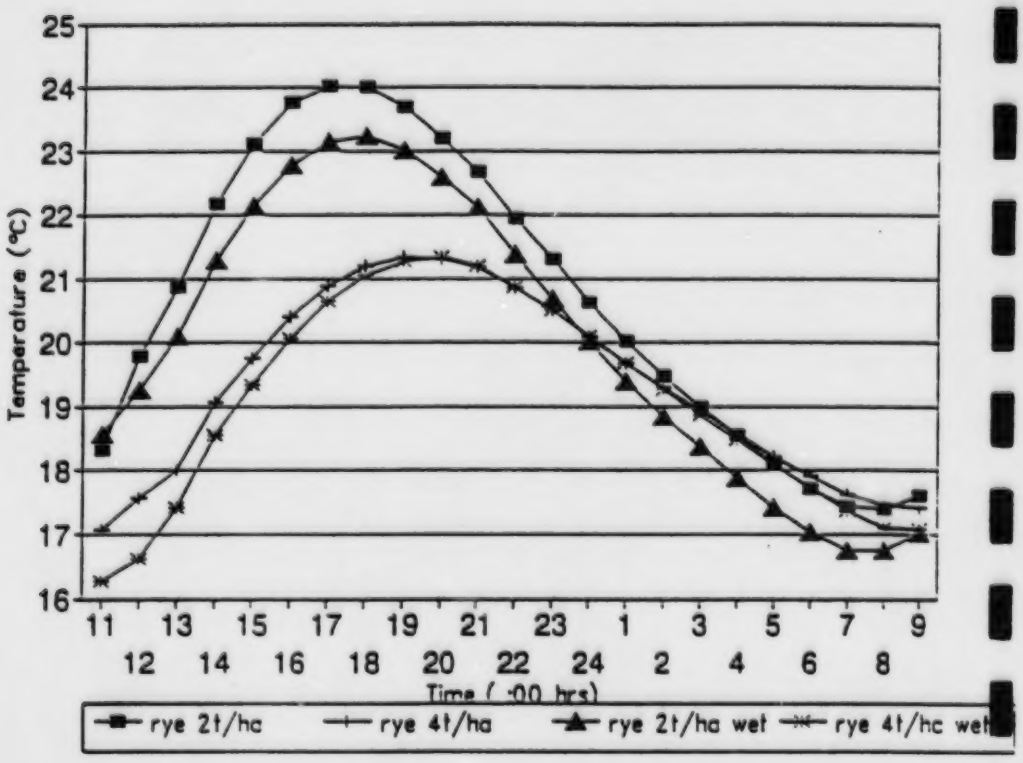



**Figure 2.4 Temperature lows for all rye treatments (June 14-15, 1988)**

Table 2.3 Final corn grain yield 1988 season.

| Treatment | Grain yield @ 15.5 % MC ($kg \cdot ha^{-1}$) |
|---|---|
| Control | 6269 |
| Control + irrigated | 6943 |
| Control + garden blanket | 6045 |
| Control + garden blanket + irrigated | 7021 |
| Corn 6 $t \cdot ha^{-1}$ | 5585 |
| Corn 6 $t \cdot ha^{-1}$ irrigated | 5661 |
| Corn 12 $t \cdot ha^{-1}$ | 5307 |
| Corn 12 $t \cdot ha^{-1}$ irrigated | 4786 |
| Rye 2 $t \cdot ha^{-1}$ | 6393 |
| Rye 2 $t \cdot ha^{-1}$ irrigated | 7385 |
| Rye 4 $t \cdot ha^{-1}$ | 6603 |
| Rye 4 $t \cdot ha^{-1}$ irrigated | 8426 |
| LSD (=0.05) | 1262 |

Table 2.4 Total, mean and average values for weather variables 1989 - Elora Research Station.

| Month | Variable | Total (1989) | Mean (1989) | Average |
|---|---|---|---|---|
| May | | | | |
| | temperature high | | 17.2 | 17.4 |
| | temperature low | | 6.2 | 5.3 |
| | rainfall | 101.8 | | 77.6 |
| | corn heat units | 312.9 | | 223.0 |
| June | | | | |
| | temperature high | | 22.1 | 23.2 |
| | temperature low | | 12.0 | 11.0 |
| | rainfall | 130.0 | | 86.9 |
| | corn heat units | 606.8 | | 594.0 |
| July | | | | |
| | temperature high | | 26.4 | 25.5 |
| | temperature low | | 13.7 | 12.7 |
| | rainfall | 8.9 | | 73.0 |
| | corn heat units | 745.5 | | 718.0 |

Table 2.5 Average plant weights and height by treatment and date.

| Treatment | Plant Weight (mg·plant$^{-1}$) | | Plant Height (cm) |
|---|---|---|---|
| | June 21 | July 14 | July 25[a] |
| corn 6 t·ha$^{-1}$ | 499 | 27400 | 160 |
| corn 12 t·ha$^{-1}$ | 452 | 24275 | 153 |
| corn 6 t·ha$^{-1}$ irr.* | 399 | 29476 | 159 |
| corn 12 t·ha$^{-1}$ irr. | 443 | 19284 | 161 |
| rye 2 t·ha$^{-1}$ | 546 | 31400 | 160 |
| rye 4 t·ha$^{-1}$ | 485 | 30425 | 169 |
| rye 2 t·ha$^{-1}$ irr. | 401 | 38452 | 151 |
| rye 4 t·ha$^{-1}$ irr. | 391 | 32524 | 162 |
| control | 635 | 30950 | 165 |
| control irr. | 510 | 33195 | 143 |
| control gb.- | 546 | 24118 | 165 |
| control gb., irr. | 470 | 26889 | 151 |
| LSD (=0.05) | 128 | 1283 | 15 |

* irrigated
\- garden blanket

Table 2.6 Final corn grain yield 1989 season.

| Treatment | Grain yield @ 15.5 % MC (kg·ha$^{-1}$) |
|---|---|
| Control | 5884 |
| Control + irrigated | 5498 |
| Control + garden blanket | 4642 |
| Control + garden blanket + irrigated | 4910 |
| Corn 6 t·ha$^{-1}$ | 5829 |
| Corn 6 t·ha$^{-1}$ irrigated | 6800 |
| Corn 12 t·ha$^{-1}$ | 5814 |
| Corn 12 t·ha$^{-1}$ irrigated | 6375 |
| Rye 2 t·ha$^{-1}$ | 6449 |
| Rye 2 t·ha$^{-1}$ irrigated | 6501 |
| Rye 4 t·ha$^{-1}$ | 6884 |
| Rye 4 t·ha$^{-1}$ irrigated | 7320 |
| LSD (=0.05) | 1286 |

# 3.0. CHAPTER 3 THE EFFECTS OF VARIOUS CROP RESIDUES AND RESIDUE PLACEMENT OPTIONS ON ZERO-TILL CORN PERFORMANCE

## 3.1. INTRODUCTION

In Ontario, zero-till grain corn (*Zea mays* L.) yields tend to be higher following crops which leave relatively low amounts of residue. Vyn (1987) reported that zero-till grain corn yields following high residue crops such as grain corn and spring killed red clover (*Trifolium pratense* L.) were often lower than those following soybeans (*Glycine max* L.), a low residue producing crop.

In zero-till systems, clearing a 10 to 15 cm band of residues from the row area has been reported to enhance rates of corn growth and development and increase grain yield compared to where residues were left in the row (Van Roestel, 1984; Vyn, 1987; Raimbault et al., 1991). Van Roestel (1984) suggested that higher corn yields where residues were cleared from the row area were due to higher in row soil temperatures compared to where residues were retained.

Yakle and Cruse [1984] reported that placement of corn residues in close proximity to corn seeds inhibited germination and early growth. They suggested that these residues released chemicals which were inhibitory to corn growth. Removal of residues from the row area prior to planting will prevent placement in close proximity to corn seeds by planter disc openers thereby reducing exposure to inhibitory chemicals.

A field study was conducted to determine the effect of various residue placement options and cropping sequences on corn growth, development, and yield in a zero-till tillage system. Corn was planted following corn which was harvested as either grain or silage, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), red clover (*Trifolium pratense* L.) which was underseeded into barley, canola (*Brassica napus* L.), soybean (*Glycine max* L. Merril) or fall rye (*Secale cereale* L.) which was seeded after soybeans. Corn was planted zero-till either with little in row residue disturbance, or after a 10 to 15 cm band of residue was cleared from the row area using planter mounted disc-furrowers. Attempts were made to correlate the presence of volatile fatty acids and phenolic acids, and differences in soil temperature, to corn performance among the different crop and residue treatments.

## 3.2. MATERIALS AND METHODS

### 3.2.1 Experimental Design

This experiment was set up as a two year rotation trial on a loam soil at the Elora Research Station. Experimental design was a randomized block split plot design with four replications. Previous crop and associated management treatments were randomly assigned to main plots and residue management treatments to subplots. The previous crops were established at various times during the initial year of the study on a site which had been fall moldboard plowed, field

cultivated twice and cultipacked prior to the planting of all crops except fall rye which was direct drilled in the fall following soybean harvest. Due to abnormally low rainfall amounts in June and July of 1988 canola, barley, and wheat residues were supplemented with residues of the same cultivar to bring residue amounts to a normal level for the Elora area. Where necessary, residues were spread evenly across the plot area in order to avoid variability in residue cover. Further description of cultural practices for each of the previous crops are presented in Appendix 1.

The main plots were 6.1 m wide by 13 m long. Previous crop and associated management treatments were as follows:

i) **Grain corn** - At physiological maturity, corn was harvested as grain using a conventional combine.

ii) **Silage corn** - By mid-September, corn was harvested as whole plant silage leaving a stubble height of 4 to 6 cm.

iii) **Hard Red Spring Wheat** - Wheat was harvested using a small plot combine and straw shredded using a rotary mower. Volunteer wheat and late season grasses were controlled using Sethoxydim and oil concentrate at a rate of 275 g $ha^{-1}$ and 7.0 l $ha^{-1}$, respectively.

iv) **Barley** - Barley was harvested as grain using a conventional combine and straw shredded using a rotary mower. Volunteer barley and late emerging grasses were controlled using Sethoxydim and oil concentrate at a

rate of 275 g $ha^{-1}$ and 7.0 l $ha^{-1}$, respectively.

v) **Early-kill Red Clover** - Red clover was underseeded into barley. All management practices for barley were similar to the barley treatment. The red clover was allowed to regrow in the spring until late April, when it was sprayed with a combination of Cyanazine, 2,4-D and oil concentrate at a rate of 2.2 kg $ha^{-1}$, 1.0 kg $ha^{-1}$, and 15 l $ha^{-1}$, respectively.

vi) **Late-kill Red Clover** - Similar to early-kill red clover except that the spray date was just prior to corn planting.

vii) **Canola** - Canola was harvested as grain using a small plot combine..

viii) **Soybeans** - Soybeans were harvested using a small plot combine and residues shredded using a rotary mower.

ix) **Early-kill Fall Rye** - Fall rye was direct drilled into soybean residues immediately after harvest. The rye was allowed to regrow until late April, when it was sprayed with Paraquat at a rate of 1.0 kg $ha^{-1}$.

x) **Late-kill Fall Rye** - Similar to early-kill fall rye except that the spray date was just prior to planting.

The subplots were four corn rows wide (3 m) by 13 m long. The residue placement treatments were:

i) **Residue removed from the row area** - A 10 to 15 cm band of surface residues were removed from the row area prior

to seed placement using ACRA-Plant Trash Whippers (ACRA-Plant Sales, Garden City, Kansas) which were mounted on the planter unit. After planting, any residues still present in the immediate row area were moved by hand. Occasionally the movement of residues was associated with movement of soil out of the row area which caused shallow seed placement. Wherever this occurred, soil was moved back into the row area to ensure proper seed depth placement.

ii) **Residue retained in the row area** - A unit-mounted ripple coulter was mounted in place of the trash-whippers in front of seed disc openers. Occasionally after red clover, the seed furrows were not completely closed by the planter. Where this occurred the furrow was closed by walking on top of the row.

**3.2.2 Second year corn management**

Corn (cv. Pioneer[1] 3902) was planted on May 16 in 0.76 cm wide rows using a model 7000 John Deere Conservation corn planter (Moline, Illinois). Starter fertilizer was applied through the planter in a band 5 cm from the row at a rate of 9.5, 38, 38 kg $ha^{-1}$ of N, $P_2O_5$, and $K_2O$, respectively. A ripple coulter was in front of the fertilizer disc openers to help ensure proper depth placement. Terbufos was applied at a rate of 10 kg $ha^{-1}$ in a band over the row where the previous crop

[1] Pioneer Hi-Bred Limited, Chatham, Ontario, Canada.

was corn. The seeding rate was 73,000 seeds $ha^{-1}$.

After planting, the herbicides cyanazine and metolachlor were applied preemergence as a tank mix at a rate of 2.0 and 2.6 kg $ha^{-1}$, respectively. In 1989, dandelions (*Taraxacum officinale* Weber) were controlled using 2,4-D at a rate of 1.0 kg $ha^{-1}$. Dicamba was applied postemergence in the 1989 growing season, at a rate of 0.60 kg $ha^{-1}$ to control broadleaf weed escapes. In early July, of the 1989 growing season, Bromoxynil was applied at a rate of 0.3 kg $ha^{-1}$ as a drop nozzle treatment to control a flush of late emerging redroot pigweed (*Amaranthus retroflexus* L.). Nitrogen was applied as a sidedress treatment in early June as Urea Ammonium Nitrate at a rate of 130 kg a.i. $ha^{-1}$. Carbaryl, an insecticide, was applied in late June of 1989, at a rate of 1.5 kg $ha^{-1}$ to control armyworm (*P. unipuncta*).

### 3.2.3 Field Measurements

Days required for 50% emergence were determined by daily monitoring 10 m of row for each plot until at least 50% of the corn had emerged. Corn plant dry matter accumulated was determined approximately 6 weeks after planting by harvesting a total of 15 plants from two adjacent rows. Corn grain yield was measured by hand harvesting after physiological maturity. Two adjacent rows, each 5 m long, were used to measure grain yield for a total of 10 m of row per plot. These same plants were later harvested to measure stover yields. All plant material was dried in ovens for at least 3 days or until they

were completely dried at 80°C.

Soil samples were taken weekly to determine the concentration of volatile fatty acids and phenolic acids starting in late April and continuing until early June and late June in 1989 and 1990, respectively. After corn was planted, soil sampling occurred within the row area. Sampling methods and laboratory procedures are presented in sections 1.1 and 1.2.

In 1989, Soil temperatures were monitored from May 25 to 31, using copper-constantan thermocouples[2] on 2 replicates. The following previous crops were monitored; grain corn, canola, soybeans, late-kill fall rye, barley, and late-kill red clover. Temperatures were recorded for both residue placement options. Soil temperatures were recorded every one-half hour throughout the day. Soil growing degree days were calculated using base temperature of 10°C as reported by Swan et al. (1987).

### 3.2.4 Statistical Analysis

All measured parameters were analyzed using an analysis of variance appropriate for a randomized complete block split plot design. Significant differences among the various previous crops and residue placement options were determined using the appropriate protected LSD at the 5% level.

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[2] Thermocouple wire from Gordon Company, Richmond, Illinois.

## 3.3. RESULTS

Days required for 50% emergence were significantly affected by previous crops and residue placement for both years (Table 3.1). In 1989, residue removal from the row area reduced days required for 50% emergence following all previous crops with differences significant following barley and early-killed red clover. However in 1990, residue removal from the row area was associated with slower rates of emergence with differences significant following grain corn, wheat, soybeans, early-killed rye and late-killed rye. In 1989, corn planted into late-killed red clover required the most days to emerge, while corn planted into early-killed rye and silage corn required the least. In 1990, days required for 50% emergence were highest following grain corn and lowest after soybeans, early-killed rye and late-killed rye.

Corn plant weights were significantly affected by previous crops in both years (Table 3.2). Plant weights were highest following soybeans and early-killed rye and among the lowest following early and late-killed red clover. In both years, plant weights after silage corn were significantly higher than after grain corn. Plant weights were affected by residue placement only in 1989 with residue removal from the row area increasing plant weights.

Plant population at harvest was not significantly affected by previous crop or residue placement in either year or when combined over years (Table 3.3).

Grain corn yields were significantly affected by residue placement in 1989, but not in 1990, when combined over all previous crops (Table 3.4). Within crop differences for residue placement were not significant in either year, but when combined over years residue removal following early-killed red clover significantly increased grain corn yields compared to where residues were retained in the row area. In 1989, residue removal increased grain corn yields following all crops by at least 400 kg ha-1 except following grain corn, late-killed red clover and early-killed rye. In 1990, grain corn yield reductions of over 400 kg ha-1 were associated with moving residues out of the row area after grain corn, canola and early-killed rye while over 500 kg ha-1 increases were associated with early and late-killed red clover.

Previous crops affected grain corn yields in both years (Table 3.5). In both years grain corn yields following grain corn were lower than silage corn and soybeans. Grain corn yields following soybeans and silage corn were similar. In 1990, grain corn yields were lower following early-killed red clover, barley and canola compared to soybeans. When combined over both years grain corn yields after canola and barley were less than soybeans.

Total above ground dry matter yields were affected by previous crops in both years (Table 3.6). Highest total dry matter yields were associated with planting corn after soybeans and lowest after grain corn.

Total dry matter yields were lower after late-killed rye compared to soybeans. In 1990 and when combined over years, total dry matter was less following silage corn compared to soybeans. Residue placement did not affect total dry matter yields.

Accumulated in row soil growing degree days (GDD) from May 25 to 31 1989 were significantly affected by residue placement but not by previous crops. Averaged across all previous crops, accumulated GDD was 54.6 and 51.8 where residues were removed and retained in the row area, respectively. Within crop differences in accumulated GDD were significant following grain corn and late-killed red clover (Fig. 3.1). Accumulated GDD were 13.6, 9.0, and 6.6 percent lower where residues were retained compared to where residues were removed from the row area for grain corn, late-killed red clover, and barley, respectively. Differences following soybeans, canola, and late-kill fall rye were less than 3%. Based upon comparisons with long term averages, the amount of sunshine (solar radiation) and air temperature were typical for the Elora area in late spring (Table 3.7). Measurable precipitation was recorded 4 out of the 7 days with a total rainfall amount of 56.9 mm.

There were no volatile fatty acids detected on any sampling day for either year.

In 1989, soil samples were taken weekly starting April 26 and continuing until June 7. Phenolic compounds were

detected on the first 4 sampling dates but none were detected within the row area after planting. The concentration of phenolic compounds tended to peak on the May 2 sample date except after soybeans and early-kill fall rye which peaked on May 9. On the day prior to planting, phenolic compound concentrations were highest following early-killed red clover at 0.6 mM. Phenolic compound concentrations were relatively high following barley, wheat, and late-killed red clover. Phenolic compound concentrations were relatively low following silage corn and early-killed rye, and negligible following soybeans and canola.

In 1990, sampling started on May 1 and continued weekly until June 26. On sampling days prior to planting, Phenolic compound concentrations tended to peak on May 8 and then decline. On the sampling day prior to planting, phenolic compound concentrations were highest following wheat at 1.6 mM. Phenolic compound concentrations were relatively high after early-killed red clover, and early and late-killed rye. Phenolic compound concentrations following grain and silage corn were relatively low and negligible after soybeans and canola.

After planting, trace amounts of phenolic compounds were measured on May 22 and May 29 following a number of previous crops. On June 5, phenolic compound concentration of 0.6 mM was measured following late-killed red clover where residue was retained in the row area. Phenolic compounds were not

detected on June 12 and June 19.

On June 26, phenolic compounds were detected following all previous crops (Table 8). Movement of residues out of the row area reduced the concentration of phenolic compounds following all previous crops with differences significant for early-killed red clover, barley, wheat and canola.

### 3.4. DISCUSSION

In 1990, abnormally cool and wet weather following planting delayed corn emergence and early growth compared to 1989. In both years, there was little effect in rankings for rates of corn emergence following the different previous crops. However, residue removal from the row area compared to where residues were retained, had opposite effects on rates of corn emergence between both years. Slower rates of corn emergence in 1990 where residues were removed compared to where they were retained in the row area may be due to soil crusting which was caused by rainfall shortly after planting.

In this study, grain corn yields were higher when planted after crops other than itself. Total removal of corn residues (silage corn) significantly increased grain corn yields compared to where residues were left (grain corn). However, clearing corn residues from the row area did not increase grain yield. Following grain corn, clearing residues from the row area was associated with lower plant populations compared to where residues were retained which may account for poor corn yields where residues were removed.

Wherever removal of residues from the row area was associated with corn yield reductions, plant populations were also lower compared to where residues were retained. After early-killed red clover, yield increases due to movement of residues out of the row area were not due to differences in plant population.

Increases in corn yield following red clover and rye which was killed 3 weeks, compared to 1 day, prior to planting were not significant.

Rankings in total above ground dry matter were similar to grain yield. Rankings for final dry matter yields were also similar to early dry matter yields with the exception of corn planted after red clover which improved relative to the other crops later in the season. The opposite occurred for corn planted after rye.

Movement of crop residues out of the row area increased in row soil GDD, which in 1989 may partially account for increased rates of corn emergence ant early growth where residues were removed. Differences in corn performance following different crops can not be explained by soil GDD.

Phenolic compounds were detected in both years indicating that crop residues are capable of releasing potentially inhibitory concentrations of phenolic acids. In 1990, significant concentrations of phenolic compounds were detected in late June, indicating that crop residues are capable of releasing phenolic compounds late in the growing

season which would be able to affect late season growth and yield. On this sample date, there was also evidence that residue removal from the row area may decrease in row phenolic compound concentrations. Improvements in corn performance where residue was removed from the row area (ie. early-killed red clover) may be due to lower phenolic acid concentrations around corn plants.

### 3.5. EFFECT OF SOIL TEMPERATURE ON THE DEVELOPMENT OF CORN GROWN IN VARIOUS CROP RESIDUES

Experiments comparing the effects of crop residues to bare controls involve various potential causes for differences in crop performance. Although crop residues can affect soil water content and nutrient availability, the main effect of residue covers is to lower soil temperature. The effect of soil temperature on corn growth and development is very important during the early plant's life cycle. During this period, any assessment of potential allelopathic effects of crop residues on corn performance should distinguish between that effect and the effect of low soil temperature on corn performance. The objective of this study was to determine to what extent the residue-related modification of soil temperature can account for differences in development between a bare control and several types of residue cover treatments during the early vegetative growth of corn.

Measurements were conducted at the Elora Research Station on the site used for Dr. Paul Voroney's research on

chemical residues associated with crop residue covers. This experiment consists of four replications of a split plot design with crop residue covers as main plots and the use or absence of use of trash whippers at planting as sub-plots. Pioneer 3902 was planted on 15 May 1990 at 72,000 plants $ha^{-1}$ using a planter equipped with or without trash whippers. Further details on the experimental layout and management can be obtained in the report of Dr. Voroney's allelopathic study (contract No. 01686-7-0339/01/SE).

Crop development was monitored from leaf 4 to leaf 8 in two of the four replicates. Soil temperature at seed depth was measured every 10 minutes and averaged hourly. The temperature readings were taken in both the non-trash whipper and trash whipper subplots of the bare and eight crop residue treatments (grain corn, spring canola, spring barley, spring barley and red clover killed early, spring barley and red clover killed late, soybean, soybean and fall rye killed early, and soybean and fall rye killed late). Four copper-constantan thermocouples connected in series to a datalogger were recorded for each treatment of one of the replicates.

Corn development is well understood and has been shown to be regulated by temperature in absence of severe soil water stress. Consequently, for each hybrid, a specific number of degree-days is required to accumulate before a leaf can emerge from the whorl. In the early part of the corn lifecycle when the apical meristem is below ground, development is driven by

soil temperature and the accumulation of degree-days at the level of the meristem. In spring, the presence of crop residues lowers soil temperature and corn development is typically slower than in a bare soil because the accumulation of soil degree-days is simply slower. On the other hand, if leaf development in a residue treatment were to be retarded by other causes in addition to low soil temperature (disease, lack of mineral nutrient, allelopathy etc...), it is expected that the number of degree-days required for leaf emergence would not only be obtained later but would also be larger than that in a bare control.

The number of degree-days required for development of corn leaves 4 to 8 in the following residue treatments (grain corn, spring barley, spring barley and red clover killed early, spring barley and red clover killed late, soybean, soybean and fall rye killed early, soybean and fall rye killed late) was similar to that of corn growing in the bare control for subplots where no trash whippers were used. There was one case where the number of degree-days required for leaves 4 to 8 to emerge was significantly higher than that of the bare treatment: spring canola required significantly more degree-days (at the 0.05 level) than the bare treatment. When this period was examined more closely, it was found that the corn plants growing in canola residue developed normally from emergence of leaves 4 to 6 but required 27.4% more degree-days than the bare control from leaf 6 to 8.

When similar types of comparisons were made for corn planted in residue treatments using a planter equipped with trash whippers, the significant difference between spring canola and the bare treatment disappeared. As with no trash whippers, spring barley, grain corn, spring barley and red clover killed early, spring barley and red clover killed late, soybean, soybean and fall rye killed early, soybean and fall rye killed late did not result in degree-days intervals significantly higher than that of the bare treatment. It must be mentioned that the two replicates, of the soybean and fall rye killed late, did not behave consistently. In one of two replicates, corn required more degree-days (at the 0.05 level) than the bare control for development of leaves 4 to 5. The average of the two replicates does not result in a number of degree-days for leaf emergence that is significantly different from the bare control. However, this discrepancy between plots indicates that this treatment may require further study even though its effect on corn development is less consistent than that of canola.

### 3.6. CONCLUSION

In conclusion, this one-year study indicates that two types of residue covers modify corn development beyond what is expected from thermal effects. This conclusion should be verified over years. However, according to this 1990 phenological analysis, spring canola is the residue type that most warrants further research on potential allelopathic

chemicals. The physical removal of canola residues from the row did alleviate the problem. If this is a case of allelopathy, it might be an indication that the allelopathic compound(s) is (are) not very volatile and/or not very mobile in the soil solution. This information should serve as a basis for the planning of further experiments on the allelopathic potential of crop residues on corn in Ontario.

Table 3.1 Effects of previous crop and residue placement on days required for 50% emergence.

| Previous Crop | Year | | | | | |
|---|---|---|---|---|---|---|
| | 1989 | | | 1990 | | |
| | Residue removed | Residue retained | mean | Residue removed | Residue retained | mean |
| | days | | | | | |
| Grain Corn | 11.8 | 12.2 | 12.0b+ | 24.8 * | 22.8 | 23.9a+ |
| Silage Corn | 10.1 | 10.8 | 10.5e | 22.6 | 22.6 | 22.6b |
| Wheat | 11.3 | 12.2 | 11.8bc | 23.2 * | 21.8 | 22.6b |
| Canola | 11.3 | 11.6 | 11.5bcd | 22.6 | 21.5 | 22.1b |
| Barley | 10.8 * | 12.6 | 11.7bcd | 21.7 | 22.0 | 21.9bc |
| Early-Kill Red Clover | 10.8 * | 13.0 | 11.9bc | 22.5 | 22.5 | 22.5b |
| Late-Kill Red Clover | 12.7 | 13.6 | 13.2a | 22.2 | 22.5 | 22.4b |
| Soybeans | 10.7 | 11.5 | 11.1cde | 21.6 * | 20.3 | 21.0cd |
| Early-Kill Rye | 11.6 | 12.3 | 12.0b | 21.6 * | 20.3 | 21.0cd |
| Late-Kill Rye | 10.7 | 11.1 | 10.9de | 22.0 * | 19.8 | 20.9d |
| Residue mean | 11.2 * | 12.1 | | 22.5 * | 21.7 | |

+ Within column means followed by the same letter are not significantly different at the 5% level.

* Within row means separated by this sign are significantly different at the 5% level using the appropriate LSD.

Table 3.2 Effect of previous crops and residue placement on corn dry weight in late June.

| | Year | |
|---|---|---|
| Previous Crop | 1989 | 1990 |
| | ----kg ha$^{-1}$---- | |
| Grain Corn | 154b+ | 51e |
| Silage Corn | 194a | 109ab |
| Wheat | 127bc | 69de |
| Canola | 122bc | 52e |
| Barley | 130bc | 86bcd |
| Early-Kill Red Clover | 116c | 67de |
| Late-Kill Red Clover | 100c | 51e |
| Soybeans | 193a | 121a |
| Early-Kill Rye | 206a | 96abc |
| Late-Kill Rye | 216a | 58e |
| Residue Placement | | |
| Removed | 166a | 77a |
| Retained | 146b | 75a |

+ Within column means followed by the same letter are not significantly different at the 5% level.

Table 3.3 Effects of previous crop and residue placement on corn plant population at harvest.

| | Year | | |
|---|---|---|---|
| Previous crop | 1989 | 1990 | mean |
| | ---- $10^3$ pl $ha^{-1}$ ----- | | |
| Grain Corn | 62.6a+ | 59.2a | 64.4a |
| Silage Corn | 68.4a | 61.7a | 65.0a |
| Wheat | 70.1a | 64.3a | 67.3a |
| Canola | 67.3a | 62.5a | 64.9a |
| Barley | 66.4a | 64.6a | 65.5a |
| Early-Kill Red Clover | 65.6a | 66.1a | 65.9a |
| Late-Kill Red Clover | 65.1a | 64.7a | 64.9a |
| Soybeans | 69.2a | 65.8a | 67.5a |
| Early-Kill Rye | 67.1a | 66.1a | 66.6a |
| Late-Kill Rye | 69.2a | 65.1a | 67.2a |
| Residue Placement | | | |
| Removed | 69.1a | 63.2a | 66.1a |
| Retained | 66.5a | 64.9a | 65.7a |

+ Within column means followed by the same letter are not significantly different at the 5% level.

Table 3.4 Grain corn yields in 1989 and 1990 for the various previous crop and residue placement treatments.

| | Year | | | |
|---|---|---|---|---|
| | 1989 | | 1990 | |
| Previous crop | Residue removed | Residue retained | Residue removed | Residue retained |
| | ---------- | Mg ha$^{-1}$ | ---------- | |
| Grain Corn | 8.06 | 7.86 | 5.98 | 6.54 |
| Silage Corn | 9.14 | 8.78 | 7.40 | 6.98 |
| Wheat | 8.69 | 8.20 | 7.31 | 7.59 |
| Canola | 8.88 | 8.31 | 6.25 | 6.69 |
| Barley | 8.70 | 8.06 | 6.96 | 6.87 |
| Early-Kill Red Clover | 8.96 | 8.20 | 7.13 | 6.62 |
| Late-Kill Red Clover | 8.37 | 8.38 | 7.44 | 6.81 |
| Soybeans | 8.85 | 8.38 | 7.77 | 7.56 |
| Early-Kill Rye | 8.96 | 8.20 | 7.13 | 6.62 |
| Late-Kill Rye | 8.45 | 7.97 | 7.48 | 7.30 |
| Residue mean | 8.62 * | 8.22 | 7.09 | 7.07 |

* Within row means separated by this sign are significantly different at the 5% level using the appropriate LSD.

Table 3.5 Effect of previous crops on grain corn yield in 1989 and 1990 averaged over the residue placement treatments.

| Previous crop | Year | | |
|---|---|---|---|
| | 1989 | 1990 | mean |
| | ------ | Mg ha$^{-1}$ | ----- |
| Grain corn | 7.96b+ | 6.26d | 7.11d |
| Silage corn | 8.93a | 7.19ab | 8.06ab |
| Wheat | 8.44ab | 7.44ab | 7.94abc |
| Canola | 8.60ab | 6.47cd | 7.53cd |
| Barley | 8.38ab | 6.92bcd | 7.65bc |
| Early-Kill Red Clover | 8.58ab | 6.88bcd | 7.73abc |
| Late-Kill Red Clover | 8.37ab | 7.13abc | 7.75abc |
| Soybeans | 8.67ab | 7.67a | 8.17a |
| Early-Kill Rye | 8.11b | 7.47ab | 7.79abc |
| Late-Kill Rye | 8.21b | 7.39ab | 7.80abc |

+ Within column means followed by the same letter are not significantly different at the 5% level.

Table 3.6 Effects of previous crop and residue placement on above ground dry matter at harvest.

| Previous crop | Year | | |
|---|---|---|---|
| | 1989 | 1990 | mean |
| | ------ | Mg ha$^{-1}$ | ----- |
| Grain corn | 12.7b* | 11.9d | 12.3c |
| Silage corn | 14.0ab | 13.1bcd | 13.5b |
| Wheat | 13.9ab | 13.8ab | 13.8ab |
| Canola | 14.2a | 12.4cd | 13.3b |
| Barley | 13.7ab | 12.6bcd | 13.2bc |
| Early-Kill Red Clover | 14.3ab | 13.0bcd | 13.6abc |
| Late-Kill Red Clover | 14.0ab | 13.6bc | 13.8ab |
| Soybeans | 14.1a | 14.9a | 14.5a |
| Early-Kill Rye | 13.8ab | 13.6abc | 13.7ab |
| Late-Kill Rye | 13.4ab | 13.7abc | 13.5b |
| Residue placement | | | |
| Removed | 14.3a | 13.3a | 13.7a |
| Retained | 13.5a | 13.2a | 13.4a |

+ Within column means followed by the same letter are not significantly different at the 5% level.

Table 3.7 Meteorological data for the period when soil temperatures were measured and long term averages for May and June at the Elora Research Station.

| Meteorological Parameter | Daily Average | | |
|---|---|---|---|
| | Sample Period | May+ | June+ |
| Solar Radiation (MJ $m^2$) | 21.0 | 18.7 | 21.7 |
| High Temperature (°C) | 21.6 | 17.4 | 23.2 |
| Low Temperature (°C) | 8.3 | 5.3 | 11.0 |

+ Long term daily averages for the month.

Table 3.8 Phenolic compound concentration as affected by residue placement following various previous crops on June 26 1990.

| Previous Crop | Residue retained | | Residue removed |
|---|---|---|---|
| | Mm | | |
| Grain corn | 0.6 | | 0.2 |
| Silage corn | 0.1 | | 0.3 |
| Wheat | 1.6 | * | 0.2 |
| Canola | 1.5 | * | 0.2 |
| Barley | 1.3 | * | 0.0 |
| Early-kill red clover | 1.8 | * | 0.0 |
| Late-kill red clover | 1.9 | | 1.2 |
| Soybeans | 1.0 | | 0.0 |
| Early-kill rye | 0.7 | | 0.4 |
| Late-kill rye | 0.7 | | 0.3 |

* Within row means separated by this sign are significantly different according to a LSD test at the 5% level.

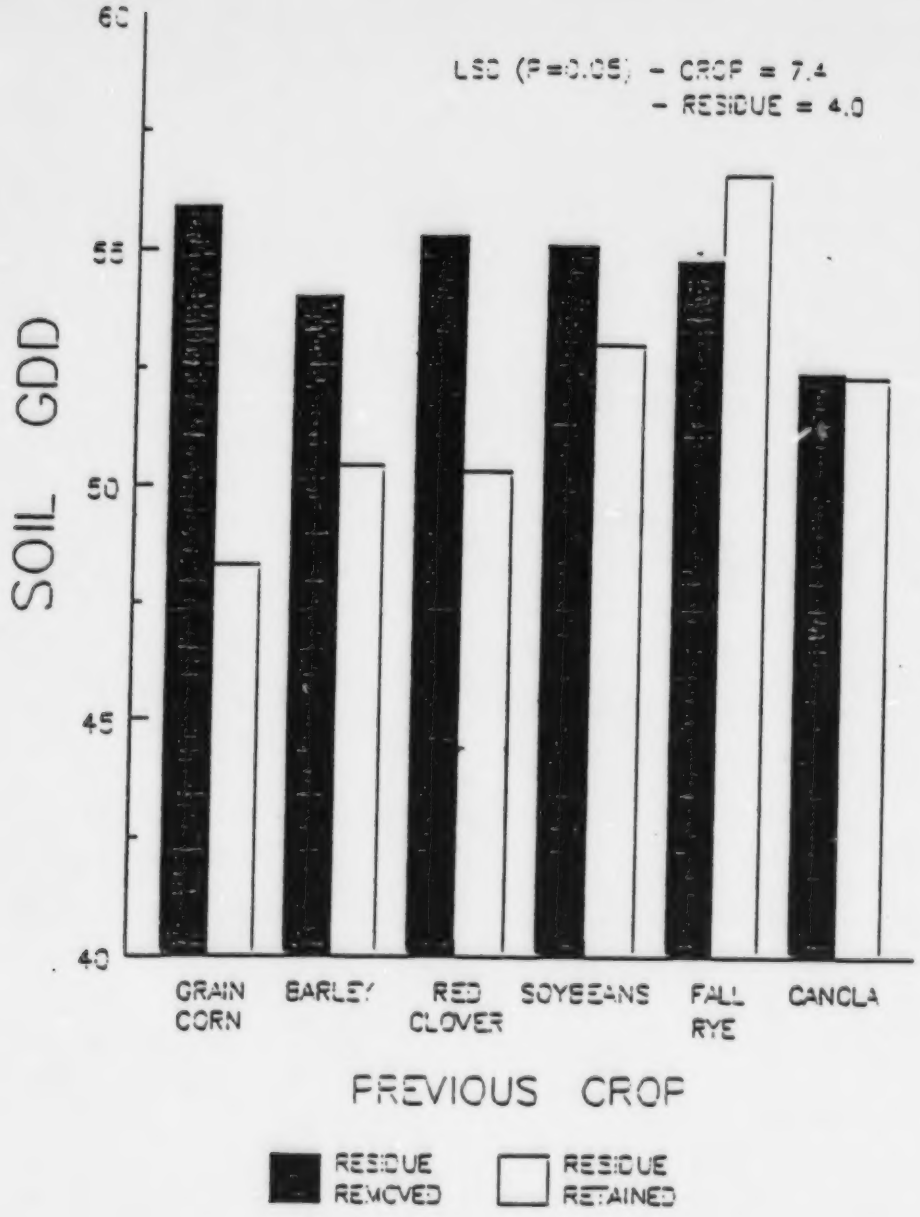


**Figure 3.1** In row soil growing degree days accumulated from May 25 to 31 between residue placement treatments following various previous crops.

# 4.0. CHAPTER 4 FIELD EXPERIMENT 1990 - CALCIUM NITRATE AMENDMENTS TO SEED ROW USING CORN AS THE TEST CROP

## 4.1. INTRODUCTION

Previous research in the laboratory has studied the effects of calcium nitrate ($Ca(NO_3)_2$) on the production and accumulation of volatile fatty acids (VFAs) and phenolic compounds (PC) during decomposition of crop residues. $Ca(NO_3)_2$ significantly reduced both accumulations of VFAs and PCs (Farquharson et al. 1990). Nitrate in the $Ca(NO_3)_2$ amendment was suspected of promoting the oxidation of the products of residue decomposition via denitrification, by acting as an electron acceptor when $O_2$ was limiting.

## 4.2. OBJECTIVE

The objective of this research was to evaluate in the field the effect of $Ca(NO_3)_2$ on phytotoxin production and accumulation during decomposition of rye (green manure) residues.

## 4.3. METHOD AND MATERIALS

### 4.3.1 Experiment Design

This experiment was a completely randomized design consisting of 8 treatments and 4 replications. The plot area had been previously cropped to soybeans and ploughed in the fall. In the latter part of May, the plot was cultivated, and fertilizer was broadcasted and incorporated to obtain a uniform fertility regime. Each plot was 3m X 15m allowing 4

corn rows to be planted. Rye residues (green manure) were placed on the surface evenly over selected plots at a rate of 6.6 dry $t \cdot ha^{-1}$ and rototilled into the surface soil. This was done to increase soil-residue contact, thus increasing the possibility of phytotoxin production during germination.

The treatments consisted of 2 corn varieties: Pioneer 3902 and Cargill 3477. The Cargill seed had been coated with Captan (N(trichloromethyl) thio-4-cyclohexene-1,2-dicarboximide). The purpose of this seed treatment was to protect the seed during germination from the phytotoxicity of herbicide residuals.

The treatments were as follows:

CC - Cargill control
CR - Cargill + rye residues

PC - Pioneer control
PR - Pioneer + rye residues
PR20 - Pioneer + rye residues + 20 kg $N \cdot ha^{-1}$ as $Ca(NO_3)_2$
PR40 - Pioneer + rye residues + 40 kg $N \cdot ha^{-1}$ as $Ca(NO_3)_2$
P20 - Pioneer + 20 kg $N \cdot ha^{-1}$ as $Ca(NO_3)_2$
P40 - Pioneer + 40 kg $N \cdot ha^{-1}$ as $Ca(NO_3)_2$

The corn was planted using a zero till planter at a rate 60,000 $plants \cdot ha^{-1}$ on May 29th.

Composite samples of soil were taken to a 5 cm depth (10 $probes \cdot plot^{-1}$) close to the planted row. Samples were taken on May 31st, June 7th, 14th, 21st, and 28th.

Water extracts of the soil were prepared using methodology similar to that described for previous research for analysis of VFAs, PCs and moisture content. Other

measurements consisted of ammonium-N and nitrate-N, plant population (July 12th) and final grain yield (15.5 % moisture).

### 4.3.2 Weather

Variables describing the weather over the growing season are reported in Table 4.4. Mean temperature highs and lows for the three months followed closely to the long-term seasonal temperatures. Rainfall was 20 mm higher than the average.

## 4.4. RESULTS

### 4.4.1 VFAs and PCs

Measurements of VFAs and PCs were at the extreme lower limits of analysis for the instrumentation. Concentrations of acetic, propionic and butyric acids and total phenolic compounds were not detectable.

### 4.4.3 Ammonium and Nitrate N

The ammonium concentration was < 2 ppm for all treatments for all sampling periods. Figure 4.1 shows the concentration of nitrate N in the soil over the growing season for the Cargill treatments. The $NO_3^-$-N concentration for both treatments followed similar patterns and were not significantly different over the season.

Comparisons were made of the means ($\alpha = 0.05$) for PC versus PR, P20 versus PR20 and P40 versus PR40. The first sampling date showed a significant difference between the $NH_4^+$-N concentration of the P40 and PR40 treatments. All other comparisons were not significant different on the first sampling date or any subsequent date. The $NO_3^-$-N concentration

between the above comparisons for all sampling dates were insignificant.

### 4.4.2 Plant Population

The population count revealed the highest population was accounted for in the CC treatment (56,944) and the lowest population count in the CR treatment (54,722). This resulted in a 91% to 95% germination rate for all treatments assuming the planter planted 60 000 seeds$\cdot ha^{-1}$ . Analysis of the population count resulted in no significant difference between any of the treatments (LSD 0.05 = 5 825).

### 4.4.4 Grain Yield

Figure 4.2 shows the final grain yields. Pioneer planted into the rye had the greatest yield (6.4 t$\cdot ha^{-1}$) and CR and P40 had the lowest yield at 5.0 t$\cdot ha^{-1}$. The addition of rye significantly ($\alpha$=0.05) decreased Cargill grain yield but increased Pioneer grain yield. The other significant difference in yield was with the PR and P40 treatments. All other treatments were not significant.

## 4.5. SUMMARY

Application of $Ca(NO_3)_2$ with the seed were not useful for overcoming the phytotoxic affects of rye residues in the Pioneer treatment. This may be due to the already high fertility levels in the soil. The coating on the Cargill seed (Captan) was also not useful for preventing the phytotoxic effects.

Table 4.1 Total, mean and average values for weather variables in 1990 - Elora Research Station.

| Month | Variable | Total | Mean | Average |
|---|---|---|---|---|
| May | | | | |
| | temperature high | | 16.1 | 17.4 |
| | temperature low | | 4.6 | 5.3 |
| | rainfall | 87.5 | | 77.6 |
| | corn heat units | 294.3 | | 223.0 |
| June | | | | |
| | temperature high | | 22.4 | 23.2 |
| | temperature low | | 11.1 | 11.0 |
| | rainfall | 84.4 | | 86.9 |
| | corn heat units | 588.6 | | 594.0 |
| July | | | | |
| | temperature high | | 24.4 | 25.5 |
| | temperature low | | 13.3 | 12.7 |
| | rainfall | 60.8 | | 73.0 |
| | corn heat units | 718.0 | | 718.0 |

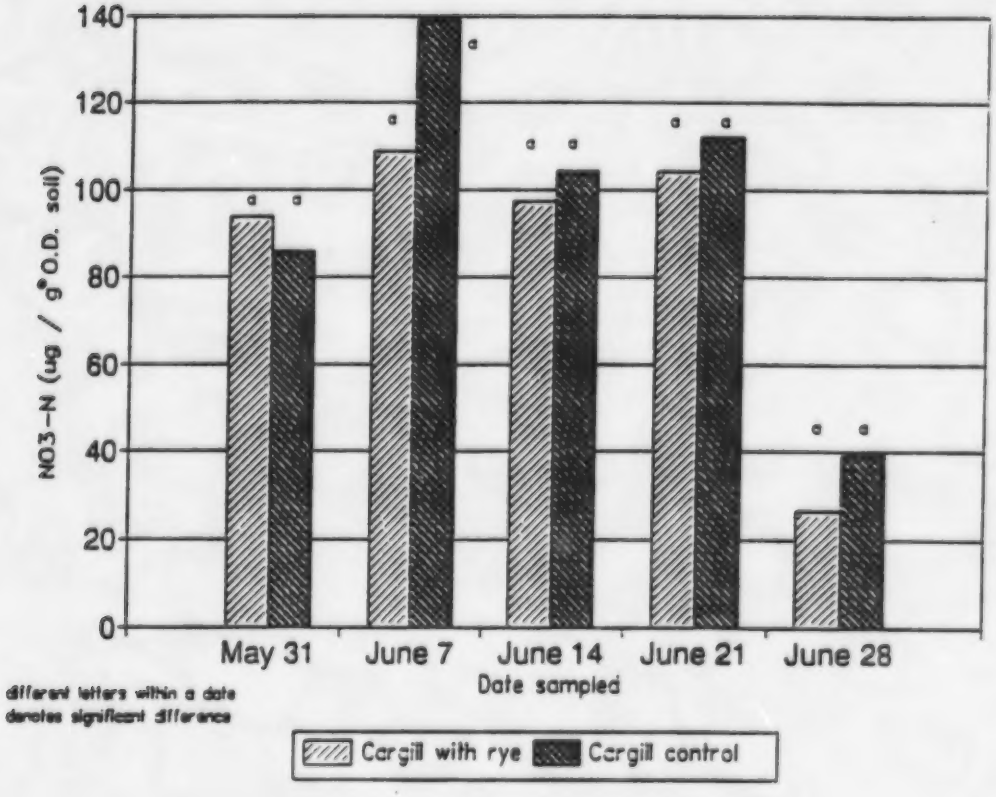


**Figure 4.1 Nitrate concentration by date for the Cargill treatments**

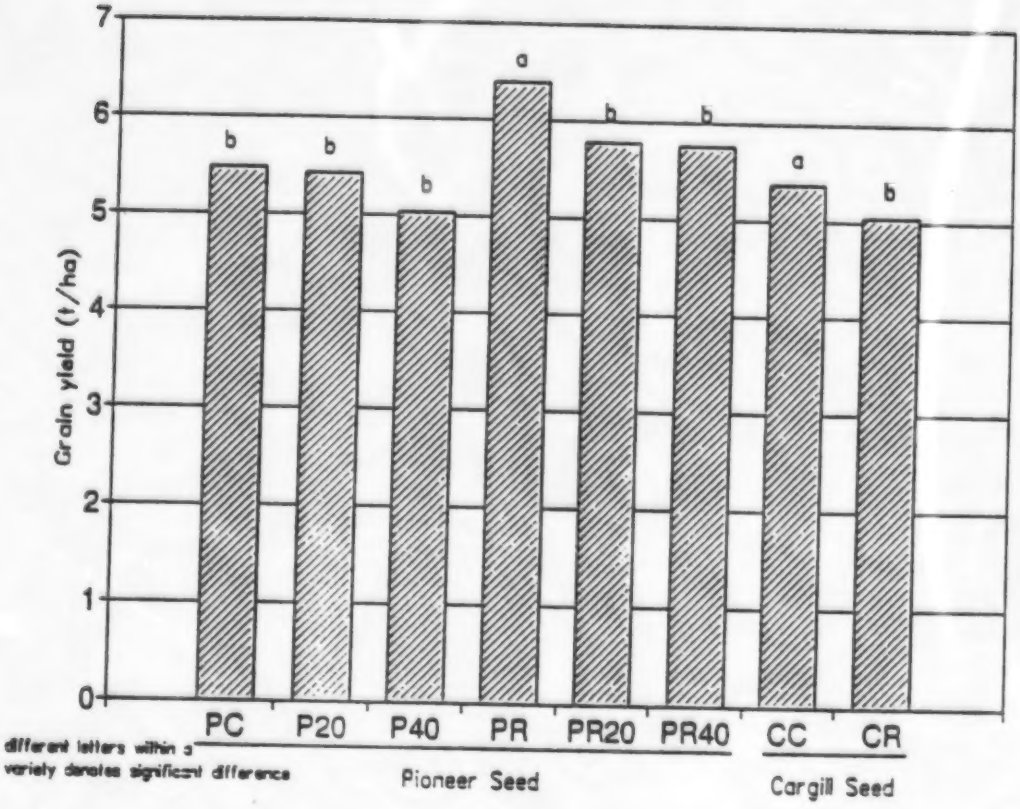


Figure 4.2 Grain yield of treated plots

## 5.0. CHAPTER 5 SATURATION VERSUS FIELD CAPACITY

The following chapters deal with studies conducted in a laboratory setting.

### 5.1. OBJECTIVE

To determine the soil environmental factors controlling the production and accumulation of phytotoxins.

### 5.2. MATERIALS AND METHODS

The study was carried out using field soil from the Elora research farm (Conestogo silt loam - air dried and sieved ≤ 2 mm), 4 residues, 2 water potentials and 3 temperatures. The following residues were used: corn stover (C), winter wheat straw (S), green rye (R) and soybean (B). The residue material consisted of the above ground stalk and leaves which was dried and finely ground. The three temperatures used in the incubation were 4°, 15° and 25°C. At the start of the incubation, an amount of water sufficient to saturate the soil (0) and to bring it approximately to field capacity (1) was added. Control treatments were comprised of soil only. The experiment was set up as a batch experiment, with sampling after 2, 5, 14, 28, 42, and 56 days of incubation.

The equivalent of 30 g oven dried soil was placed in a 250 mL glass amber bottle. The residue was added at 1% by weight and mixed throughout the soil. The bottles were sealed by use of suba seals (Suba Seal, Barnesley, England) and left sealed until sampling occurred. Measurements taken consisted of $CO_2$-C, VFAs and total phenolics.

The $CO_2$ measurement was taken using a 0.4 ml sample of bottle atmosphere and injecting into a gas chromatograph. The chromatograph is a Gowmac model 550 equipped with a column containing Porapak Q as column packing. The carrier gas is helium with a flow rate of 40 $mL \cdot minute^{-1}$. Analysis of $CO_2$ was determined by a thermal conductivity detector. Temperature settings for injection port, column and detector are 50, 70, and 70°C respectively. The bridge current setting was 190 mA.

VFAs and total PCs were determined by procedures outlined in section 1.1 and 1.2.

**5.3. RESULTS**

The green rye and more easily decomposable soybean residue showed significantly higher accumulations of $CO_2$, VFAs and PCs then the corn stover or wheat straw residues. The second trend reveals that under high temperatures the dynamics of the system occur quickly with large differences in values between sampling dates. Whereas, under the 4° temperature the dynamics reveal a constancy over time not seen in the other two temperature regimes.

The carbon dioxide measurement was taken as a curiosity so will be dealt with on a superficial level. Generally as time went from time zero to 56 d the $CO_2$ measured increased each sampling date, reaching a peak on d 14, although some of the treatments didn't reach a peak evolution until d 28, but which ever occurred, the following sampling measured a decrease. Figure 5.1 and 5.2 represent the concentration of

$CO_2$ found in the bottle atmosphere, not taking into account $CO_2$ in aqueous form, for soil amended with rye and corn incubated at 25°C. The figures also show the effect of the 2 water potential regimes. Under rye amendment the -33 kPa reveals an evolution trend which is less than the 0 kPa regime. Whereas the corn amendment reveals greater evolution during the first 14 days, levels off for sampling day 28 and 42 and dropping on the 56th day. All sampling dates were significantly different ($\alpha$ = 0.05) in $CO_2$ concentration between the rye and corn residue amendment under the 0 kPa water regime. However, under the -33 kPa regime there was no significant difference on sampling day 14 or day 42. The bean residue amendment was comparable to rye residue amendment trends as was winter wheat straw comparable to corn residue amendment trends. The effect of temperature on $CO_2$ evolution followed a general trend of reduction. Figure 5.3 represents the bean residue amendment (0 kPa) at the three incubation temperatures with comparable controls. All sampling points were significantly different with respective control points. These general trends were found throughout the study with regard to the other residue amendments.

The accumulation of VFAs also followed some general trends under the incubation criteria. Acetic acid was the most prevalent acid detected in all treatments. Peak accumulation was found no less than 14 days after start of incubation and may have occurred on day 28 depending on residue type and

incubation temperature. Generally under 0 kPa conditions the accumulation of VFAs was greater. Figure 5.4 represents the general trends found for total VFAs under 0 kPa and 25°C regimes. The green rye material and the mature bean residues had the greatest accumulation followed by mature corn stover and wheat straw. As incubation temperatures decreased the accumulation of VFAs was reduced. The easily decomposable materials (rye and bean) revealed greater differences in accumulation between sampling dates than the corn stover and wheat straw treatments especially at the 15 and 4°C temperatures. Incubations under the lower temperatures for corn stover and wheat straw tended to have small accumulations ($\leq$ 25 $\mu$g acetic-C $\cdot$ $g^{-1}$ O.D. soil) which remained steady over the sampling period. Figures 5.5 and 5.6 portray the individual VFA concentration for a given temperature and water potential for the soybean residue amendment. The higher temperature and greater water content have greater accumulation of VFAs. As temperature decreases so does the accumulation of VFAs. Under the 4°C incubation the accumulation of acetic acid reaches a steady state by the 14$^{th}$ sampling date and remains consistent over the succeeding sampling dates.

The concentration of total phenolics in the study followed slightly different trends in comparison to VFA accumulation. Total phenolics tended to appear early in the incubation sampling (Figure 5.7) and decreased in the latter

stages of the study. Rye residue under the high temperature resulted in increasing concentrations of total phenolics from the 2nd to 14th sampling day in comparison to the low temperature regime which resulted in the highest concentration occurring on day 2. The 15°C temperature followed the trend of the higher temperature with the highest concentration measured on sampling day 28. Similar trends were found for the soybean residue. The total phenolics measured from the corn stover and wheat straw residues were in the magnitude of 10 times less than total phenolics measured from the rye or soybean material. The water potential had the effect of reducing the concentration of total phenolics measured.

## 5.4. SUMMARY

The findings from this study show the effect of residue material, temperature and water potential on the evolution of $CO_2$ and concentration of VFAs and phenolics. Carbon dioxide evolution is greatest under high temperatures and residues which are easily decomposable.

Fresh, green or easily decomposable residues have the potential for accumulating the greatest concentration of VFAs and total phenolics under wet conditions in conjunction with high temperatures. Measurable quantities can be measured within 48 hours to 28 days if conditions are conducive. As moisture and temperature decreases the accumulation of phytotoxins decreases. The same trends are possible with residues which are not as easily decomposable but the

magnitude is decreased.

VFA accumulation tends to increase over time reaching a peak accumulation between 14 and 28 days of static incubation whereas total phenolic concentration tends to be higher, earlier and decreases over time. This is a result of the dynamic soil system. VFAs are by products of fermenters whereas the phenolics initially, if not totally, are of plant origin, leached from the residues.

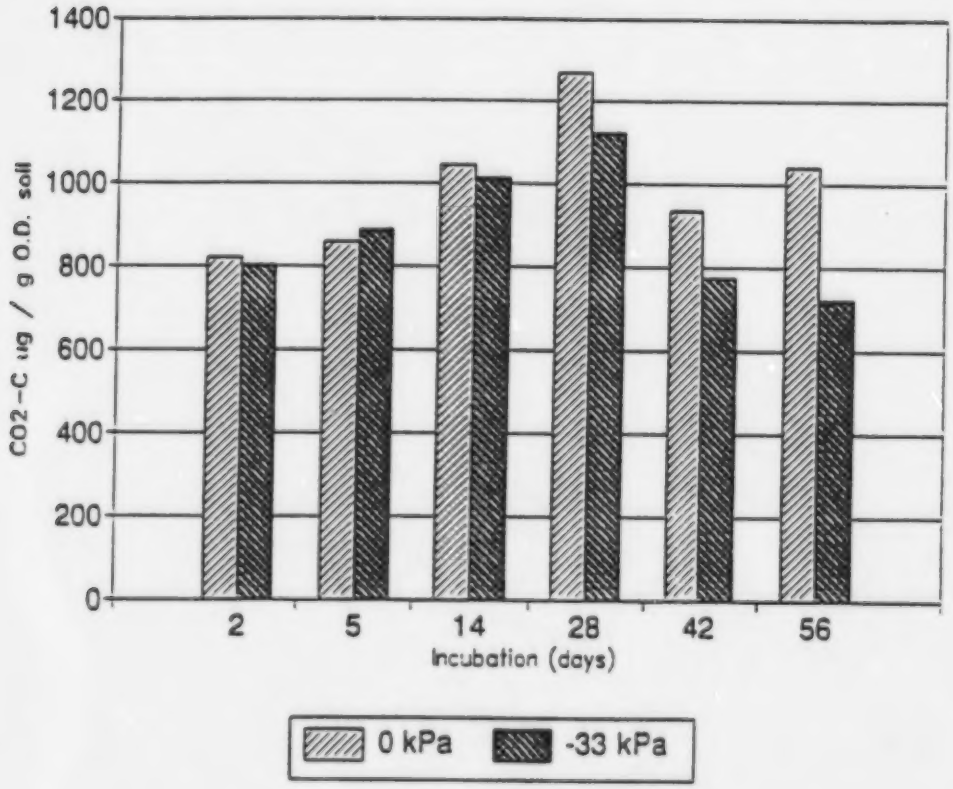


Figure 5.1 $CO_2$ concentration from 25°C incubated rye residues at 0 and -33 kPa with time.

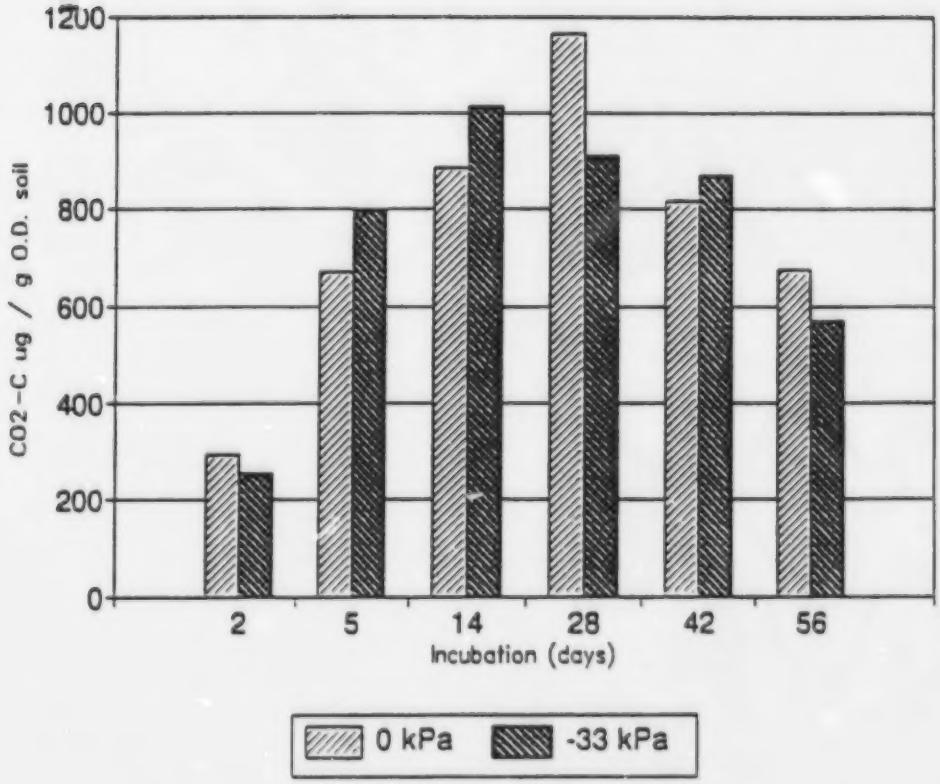


Figure 5.2 $CO_2$ concentration from 25°C incubated corn residues at 0 and -33 kPa with time.

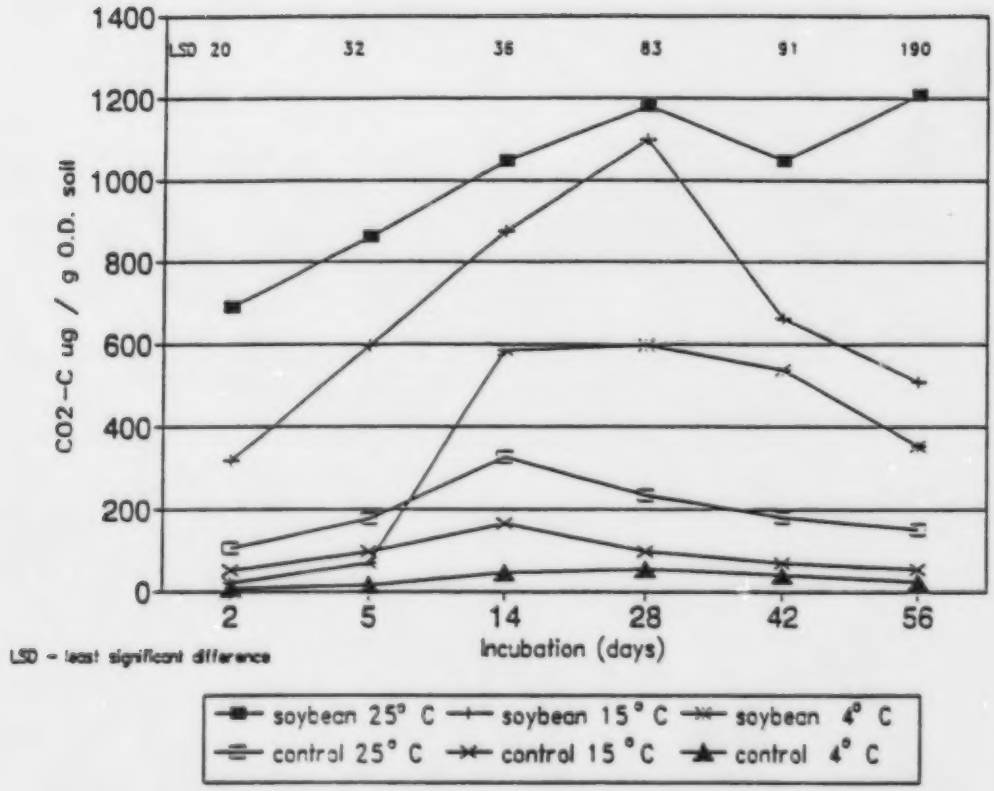


Figure 5.3 $CO_2$ concentration from incubated soybean residue at 0 kPa, at three temperatures with time versus the control treatment.

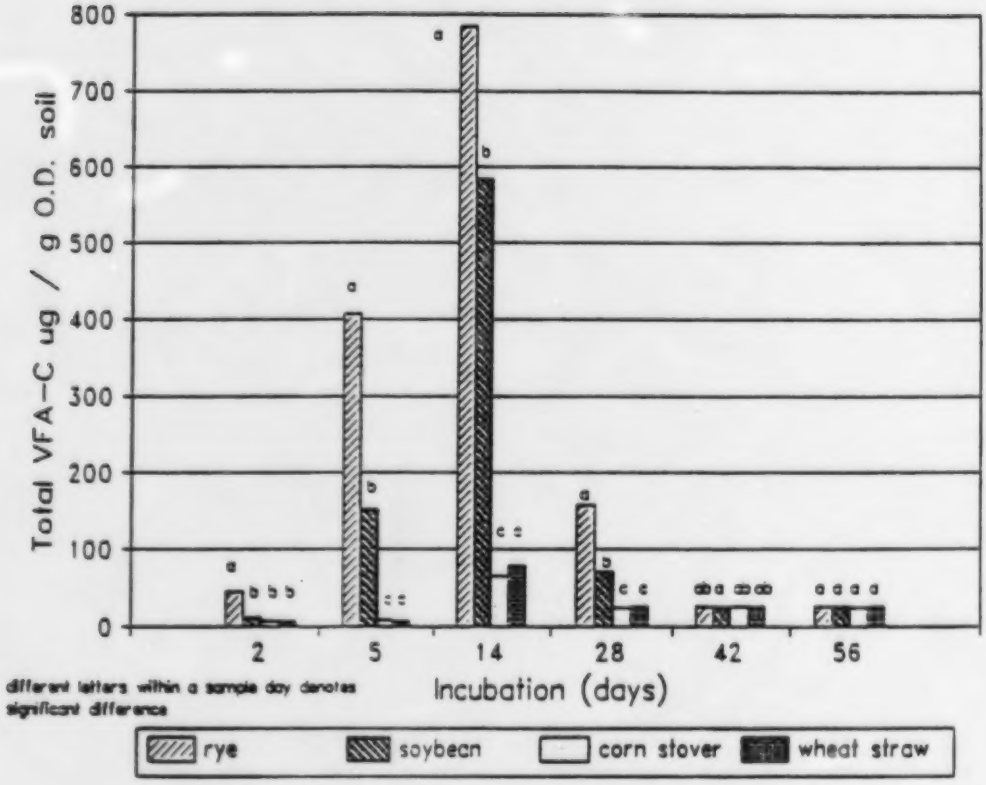


Figure 5.4 Total VFA accumulation by residue with time when incubated at 25°C and 0 kPa.

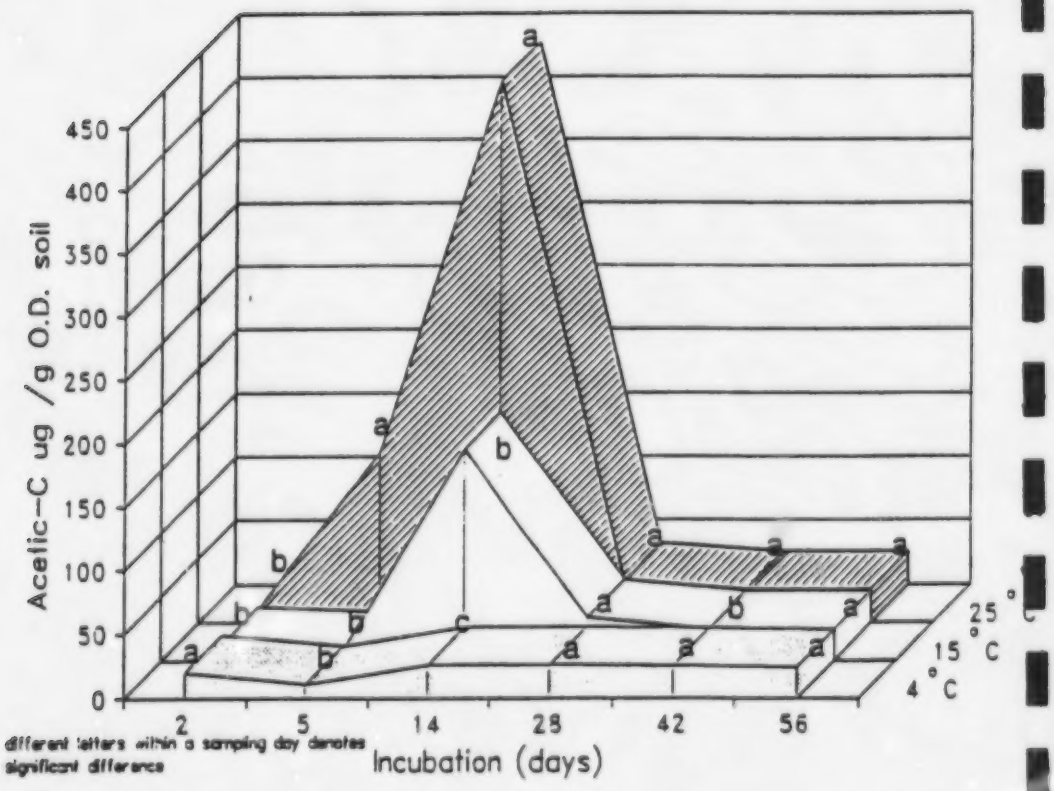


Figure 5.5 Acetic acid accumulations from soybean residue incubated at 25°C, 15°C, 4°C and 0 kPa with time.

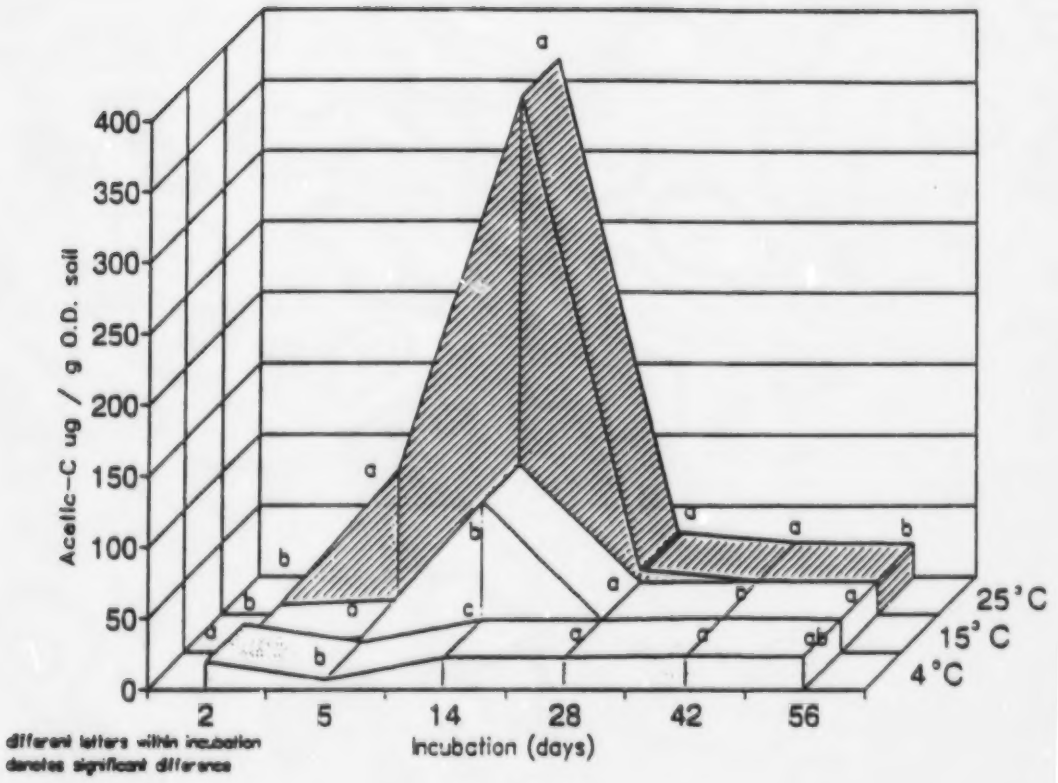


Figure 5.6 Acetic acid accumulations from soybean residue incubated at 25°C, 15°C, 4°C and -33 kPa with time.

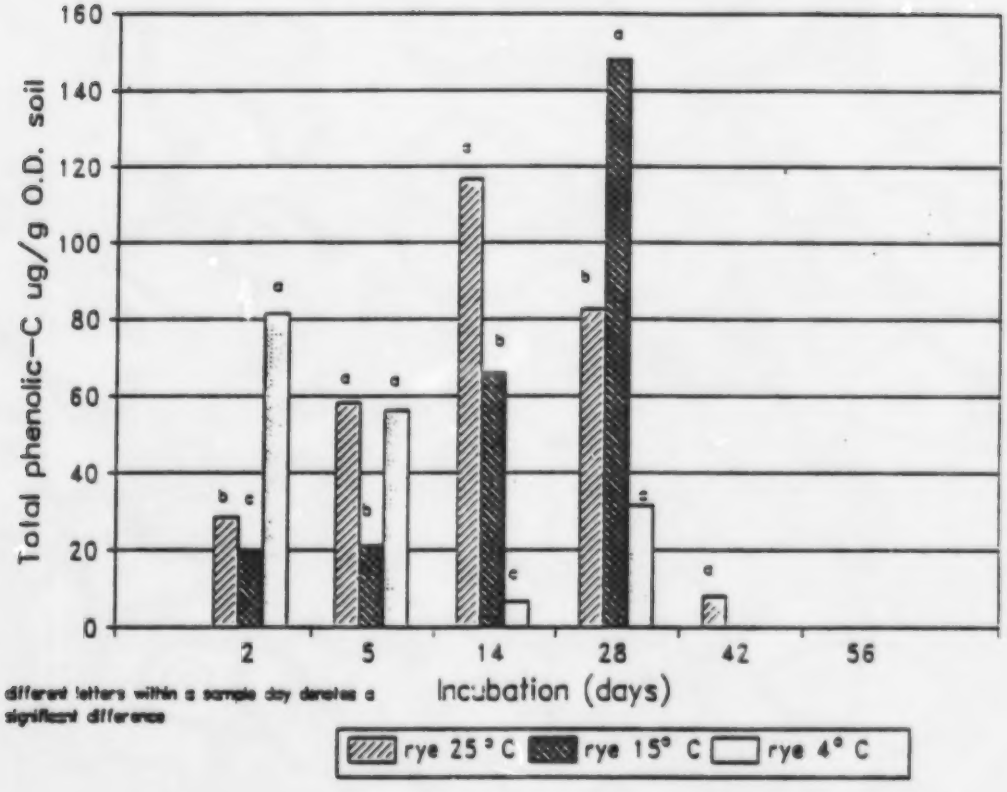


**Figure 5.7** Total phenolic acid accumulation from rye residue incubated at three temperatures and -33 kPa with time.

## 6.0. CHAPTER 6 WEATHERED versus FRESH RESIDUES

### 6.1. OBJECTIVE

To compare the production of volatile fatty acids and phenolic compounds from weathered versus fresh mature residues under saturated conditions and 15°C.

### 6.2. MATERIALS AND METHODS

The study was carried out using field soil from the Elora research farm (Conestogo silt loam - air dried and sieved ≤ 2 mm). The residues were mature alfalfa, taken prior to flowering, and corn stover and soybean straw taken at harvest. Overwintered residue of the same three materials was used as weathered material. The residue material consisted of the above ground stalk and leaves which was dried and finely ground. The temperature used in the incubation was 15°. At the start of the incubation the amount of water added was calculated to obtain a saturated state. A set of bottles with soil and water additions were used as controls. The experiment was set up as a batch experiment, due to the destructive sampling, with sampling on days 2, 4, 8, 16, and 32.

The equivalent of 20 g oven dried soil was placed in a 250 mL glass amber bottle. The residue was added at 1% by weight and mixed throughout the soil. The bottles were sealed by use of suba seals (Suba Seal, Barnesley, England) and left sealed until sampling occurred. Measurements taken consisted of $CO_2$-C, VFAs and total phenolics and were determined as outlined in sections 5.2, 1.1 and 1.2, respectively.

## 6.3. RESULTS

The general trends found in this study were that easily decomposable material resulted in higher evolution of $CO_2$, possibility of phenolic compounds and accumulation of VFAs. The ranking of $CO_2$ evolution from high to low was alfalfa, soybean and corn, respectively on all sampling dates. In all cases the amount of $CO_2$ evolved was significantly different between the three fresh residues as well as the three being significantly different from the control ($\alpha = 0.05$). Figure 6.1 depicts $CO_2$ measured from the alfalfa treatment in comparison to the control. These 3 treatments increased over time, peaked and fell off on the last sampling day thus following similar trends but resulted in different measurable quantities. The weathered treatments basically followed similar trends as above for days 2, 4 and 8. On the 16$^{th}$ and 32$^{nd}$ weathered corn had a greater amount of $CO_2$ but was not significantly different. After day 2 all treatments, whether fresh or weathered, had significantly greater measurable $CO_2$.

Measurable quantities of total phenolics occurred for fresh soybean (8.7 $\mu$g phenolic-C$\cdot g^{-1}$ O.D. soil) on day 2 and day 8 and 16 for fresh alfalfa, 98.7 and 273.2 $\mu$g phenolic-C$\cdot g^{-1}$ O.D. soil, respectively.

The accumulation of propionic and butyric acid follows closely to the total phenolic trend. Propionic acid was measurable on day 8 and 16 from the fresh alfalfa treatment. Those were the same day and treatment under which trace

amounts of butyric acid were also measured. Figure 6.2 represents the acetic-C measured from the fresh and weathered residues. There is no significant difference between any of the pairs in the first 4 days. The only significant difference comes on day 8 for the alfalfa treatments which is carried on to day 16. On day 32 there was no measurable acetic-C from any of the treatments.

## 6.4. SUMMARY

Green material has a greater potential for accumulating concentrations of compounds measured and are considered phytotoxic. Potential for phytotoxin accumulation decreases as plant residue become more mature and lignified. Therefore, the fresher and more easily decomposable the residue the greater the potential for accumulation of phenolic compounds as well as VFAs.

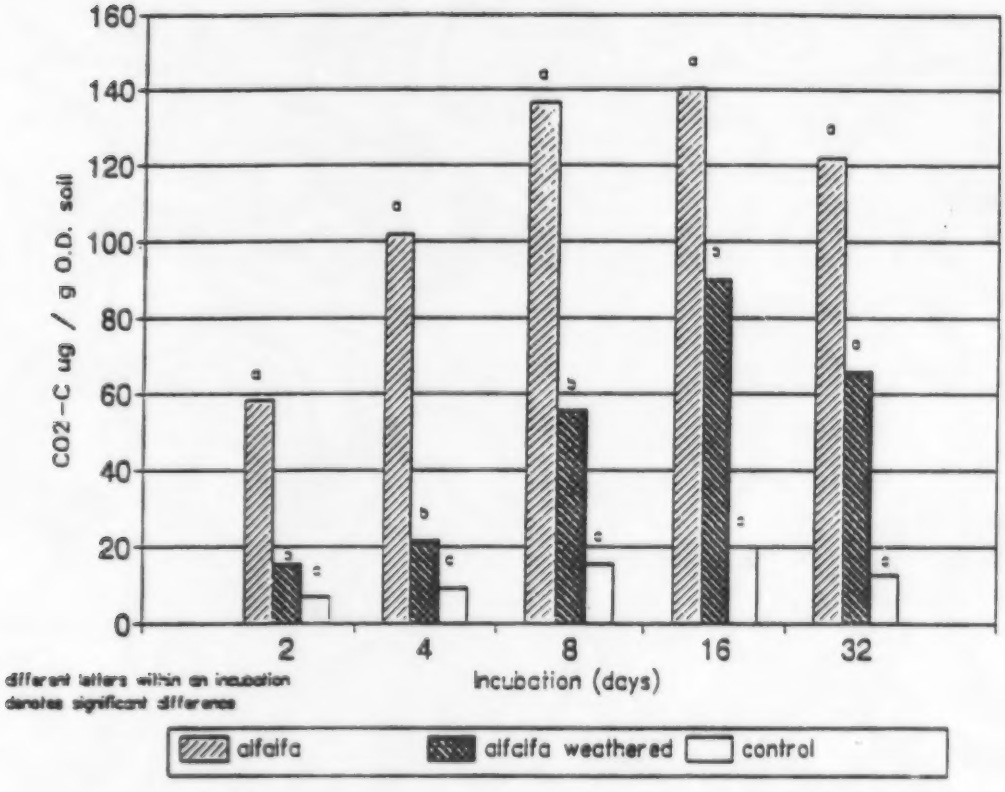


Figure 6.1 $CO_2$ concentration from fresh and weathered alfalfa amended soil versus a soil control with time.

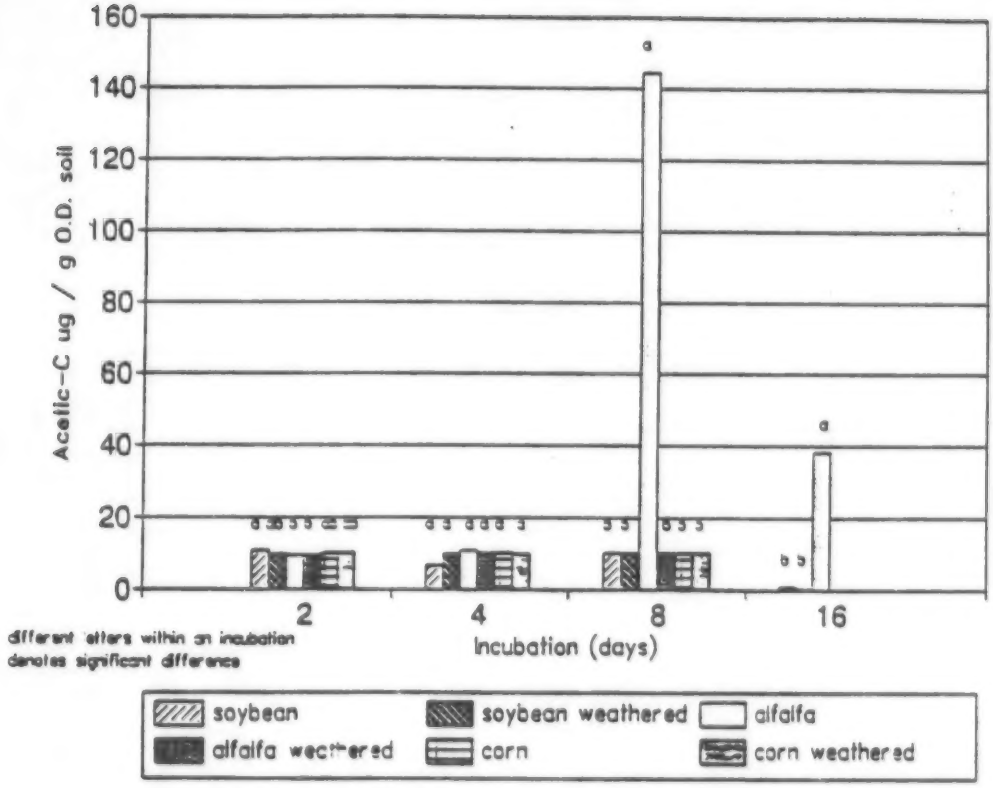


Figure 6.2 Acetic acid accumulation from fresh and weathered soybean, alfalfa and corn residues with time.

# 7.0. CHAPTER 7 EFFECTS OF VOLATILE FATTY ACIDS AND PHENOLIC ACIDS ON EARLY CORN RADICLE GROWTH

Volatile fatty acids and phenolic acids are two classes of allelochemicals which have been implicated as affecting corn performance. A series of bioassays were conducted to determine if either volatile fatty acids or phenolic acids have an effect on early corn growth. The bioassays were conducted with solutions adjusted to different pH levels to determine if acidity has an effect on corn response to the presence of these acids. Three corn hybrids were included in these bioassays to determine if a difference in hybrid response to these acids exists.

## 7.1. MATERIALS AND METHODS

Solutions containing various volatile fatty acids (acetic, propionic, and butyric) and phenolic acids (*p*-coumaric, *p*-hydroxybenzoic, ferulic, and vanillic) were prepared using double distilled deionized water. Concentration levels for the volatile fatty acids were 0.0 M (control), 0.0001 M, 0.001 M, 0.005 M, and 0.01 M. while for phenolic acids they were 0.0 M (control), 0.000001 M, 0.00001 M, 0.0001 M, and 0.001 M. Bioassays were conducted with the solutions adjusted to pH levels of either 4.5 or 7.5 using either potassium hydroxide (KOH) or hydrochloric acid (HCl). Corn hybrids used were Pioneer[3] 3737, Pioneer 3949, and

[3] Pioneer Hi-Bred Limited, Chatham, Ontario, Canada

Hyland[4] 2260.

Bioassays were conducted on an individual acid basis and in combinations. The following combinations (acetic-propionic, acetic-butyric, coumaric-vanillic, and coumaric-ferulic) were used to determine if they affected corn radicle growth. Acid combinations were mixed in a 3:1 molar ratio with the first acid mentioned in the above combinations present in a molar concentration 3 times higher than the second. These acids were mixed such that their additive molar concentrations equalled the concentration levels used in the individual acid bioassays.

A germination test procedure recommended for corn germination testing by Agriculture Canada (1979), commonly referred to as the roll-towel germination test, was used. In this method, 22.5 by 27.5 cm regular weight (38 lb) Anchor Seed germination paper (Anchor Paper, St Paul, MN) was briefly soaked in the various solutions. Germination paper was soaked by passing each sheet through the solution which was in a flat glass tray and was allowed to drag over the lip of the tray to remove excess solution. Three sheets of paper were used per roll-towel.

After the sheets were soaked, 2 of the 3 sheets were laid out flat onto a table on top of each other. Ten seeds were then placed about 2 cm from the top edge (27.5 cm edge). The third sheet was then placed on top of the seeds and other

[4] Hyland Seeds, Blenheim, Ontario, Canada.

sheets and pressed firmly to remove wrinkles and ensure good seed-paper contact. The papers were then loosely rolled (about 6 turns) with seeds remaining at the top and then sealed in a plastic bag. They were then placed in a dark growth cabinet set at a constant 25°C in an upright position for three days.

### 7.1.1 Statistical analysis

Data was analyzed using average length (cm) of ten radicles per towel; seeds that did not germinate were given a radicle length of zero. Averaged lengths were then divided by the average control radicle length for respective levels of block, pH and hybrid. Radicle lengths are expressed as percent of the control (double distilled-deionized water).

All acid and acid combinations were analyzed individually as a three level factorial which was repeated twice using SAS version 6.03 ANOVA procedure (SAS Institute Inc., 1988). Determination of differences among various acids were conducted by analyzing all phenolic and volatile fatty acid bioassays in two combined analyses in a split-plot type of design where acids were treated as main plots and various treatment levels within acids as subplots. Differences among various means were determined using appropriate LSD's at the 5% level.

In acid combination bioassays, the following formula was used to determine if various combinations were either synergistic, additive, or antagonistic to early corn radicle

growth: Combination (A,B) - 1/2(A + B). In this formula combination (A,B) is percent radicle length at the high concentration, while A and B are percent radicle lengths for the respective acids present in the individual bioassays at concentrations which they were present at in the mixture. Effects of acids when in combination were either synergistic, additive, or antagonistic if the number derived from the above formula was either statistically less than, equal to, or greater than zero, respectively.

Significance of values derived from the equation were determined using a two-tailed t-test. Standard errors used in the t-tests were derived by pooling residual errors from the combination and two individual acid bioassays. Values for individual acids in the formula were estimated by linear interpolation between the two closest concentration levels, since concentrations in which these acids were present in combination bioassays were not present in the individual bioassays.

## 7.2. RESULTS

### 7.2.1 Volatile Fatty Acids

Sources of variation for analyses of variance and their significance for individual volatile fatty acids (VFA) and VFA combination bioassays are presented in Table 7.1. In these bioassays, the source of variation referred to as block is the variation which could be accounted for by repeating the

experiment twice. In all bioassays, except for acetic-butyric, there was a significant block effect indicating that average radicle growth over all concentration, pH, and hybrid levels was not consistent between both blocks. Two way interactions between block and pH (A X B), concentration (A X C), and hybrid (A X D) were often significant indicating that between blocks the response was not consistent for the various treatment sources of variation.

Response of radicle growth to concentration levels for the various acids was significant with inhibition of radicle growth relative to the control significant at 0.001 M for acetic, propionic, and butyric ; at 0.005 M for acetic-butyric ; and 0.01 M for acetic-propionic (Fig. 7.1). The magnitude of inhibition at concentration 0.01 M was butyric > propionic > acetic = acetic-butyric = acetic-propionic.

Variation in radicle lengths caused by adjusting pH of solutions to either 4.5 or 7.5 was significant for acetic, propionic and acetic-butyric (Table 7.1). Where significant effects occurred, the inhibition of growth was greater at pH 4.5 than at 7.5 (Table 7.2). Within pH rankings of inhibition for acetic, propionic and butyric acids were the same (Table 7.2). However acetic-propionic was less inhibitory at pH 4.5 than acetic-butyric, while the opposite occurred at pH 7.5. At both pH levels acetic-propionic and acetic-butyric combinations tended to be less inhibitory than the individual acids.

The pH by concentration interaction was significant for all bioassays except for acetic acid (Table 7.1). In the propionic and acetic-butyric bioassays, inhibition of radicle growth was greater at pH 4.5 compared to 7.5 and increased as concentration increased (Fig. 7.2). In the butyric acid bioassays there was no difference in radicle growth for any concentration except at 0.0001 M where inhibition at pH 4.5 was greater compared to 7.5 (Fig. 7.2). The interaction for the acetic-propionic bioassay was due to significantly greater inhibition of radicle length at pH 4.5 at 0.0001 M, but significantly less inhibition at 0.001 M and 0.005 M compared to pH 7.5 (Fig. 7.2).

In propionic, butyric and acetic-butyric bioassays, differences among hybrids were significant (Table 7.1). In the propionic and butyric bioassays, inhibition of Hyland 2260 and Pioneer 3949 was significantly greater than Pioneer 3737 (Table 7.3). However, the opposite occurred in the acetic-butyric bioassay. Rankings among hybrids were similar for individual acids (Table 7.3). However in the acid mixtures, Pioneer 3737 was inhibited more by acetic-butyric than acetic-propionic while the opposite occurred for Pioneer 3949 (Table 7.3).

A pH by hybrid interaction was significant for propionic and butyric acids (Table 7.1). Significant reduction in radicle lengths for Pioneer 3949 at pH 4.5 relative to 7.5 was responsible for the interaction in propionic acid bioassays

(Table 7.4). In butyric acid bioassays a significant reduction in radicle lengths for Pioneer 3737 at pH 7.5 relative to 4.5 was responsible for the interaction.

In the propionic and butyric acid bioassays, there was a significant concentration by hybrid interaction (Table 7.1). There was a tendency for differences in percent radicle lengths not to be significantly different until the 0.005 M concentration, where the rate of inhibition of Pioneer 3737 was less than in the other two hybrids (Fig. 7.3). Differences in inhibition at higher concentrations caused most of the hybrid differences for these two acids.

The three way interaction between pH, concentration, and hybrid was not significant for any VFA bioassay (Table 7.1).

There was no evidence that acetic-propionic and acetic-butyric acids combinations inhibited corn radicle growth in a non-additive manner (Table 7.5).

### 7.2.2 Phenolic acids

Sources of variation for analyses of variance and their significance for individual phenolic acid and phenolic acid combination bioassays are presented in Table 7.6. Significant variations due to blocking the experiment occurred for vanillic, hydroxybenzoic, ferulic, and coumaric bioassays (Table 7.6). Two way interactions between block and pH (A X B), concentration (A X C), and hybrid (A X D) were often significant indicating that corn radicle growth between the various treatments and blocks was not consistent.

Variation caused by different levels of concentration were significant for vanillic and coumaric acids, and for the coumaric-vanillic combination. Where significant differences occurred, inhibition was significant only at 0.001 M relative to the control (Fig. 7.4). Differences in inhibition among the various acids were not significant at the 5% level for any concentration.

Inhibition of radicle length was significantly greater in hydroxybenzoic and coumaric-vanillic bioassays at a pH of 4.5 compared to 7.5 (Table 7.7). Differences in inhibition for the various phenolic acids were significant within pH 4.5 with coumaric-vanillic and coumaric-ferulic being the most and least inhibitory, respectively. However, differences in inhibition were not significant within pH 7.5. The pH by concentration interaction was not significant for any phenolic bioassay (Table 7.6).

Variation in radicle lengths for the different hybrids were significant for ferulic and coumaric acids and the coumaric-ferulic acid mixture (Table 7.6). In each case, Pioneer 3737 was inhibited more than Hyland 2260 (Table 7.8). Differences among the various acids were significant within each hybrid (Table 7.8). Generally, coumaric acid and coumaric-vanillic acid combination were the most inhibitory for all hybrids, while vanillic and hydroxybenzoic acids were the least inhibitory.

Hybrid by pH interaction was significant for vanillic

and coumaric acids and for the coumaric-ferulic and coumaric-vanillic combinations (Table 7.6). In vanillic and coumaric-vanillic bioassays, the interaction occurred where Hyland 2260 was inhibited more at pH 4.5 compared to 7.5 (Table 7.9). A similar effect occurred in coumaric bioassays except that Pioneer 3737 was inhibited more at pH 4.5 compared to 7.5. In coumaric-ferulic bioassay the interaction occurred when Pioneer 3949 was inhibited more at pH 7.5 compared to 4.5.

Concentration by hybrid interaction was not significant for any phenolic bioassay performed (Table 7.6). The three way interaction between pH, concentration, and hybrid was significant only in the coumaric acid bioassay (Table 7.6). This interaction was due to a significant reduction of radicle growth in Pioneer 3737 at 0.00001 M and 0.0001 M at pH 4.5 compared to 7.5 (Fig. 7.5).

Phenolic acids in combination appeared to affect corn seedling radicle growth in a non-additive manner (Table 7.10). Coumaric and ferulic acids in combination were antagonistic, while coumaric and vanillic acids were synergistic to corn radicle growth.

## 7.3. DISCUSSION

In all bicassays except for acetic-butyric, coumaric-vanillic and coumaric-ferulic combinations, a significant block effect occurred (Table 7.1 and 7.6). Differences in growth cabinet temperatures, and length of time between initiating the bioassay and taking measurements (72 ± 2

hours) may have interacted in causing variations in radicle length between blocks and two-way interactions with block.

These bioassays indicate that both volatile fatty acids and phenolic acids are inhibitory to early corn radicle growth. Within the range of common concentrations tested, there was very little difference in the amount of inhibition caused by volatile fatty acids compared to phenolic acids. Greater inhibition of radicle growth in volatile fatty acid bioassays occurred at concentrations which were higher then in phenolic acid bioassays.

Phenolic acids have been extracted from soils in concentrations ranging from $7 \times 10^{-4}$ to $4.9 \times 10^{-3}$ M (Whitehead, 1964). Guenzi and McCalla (1966) also extracted phenolic acids ranging in concentrations from 1 to 14 ppm, calculated on an oven-dried soil basis. Assuming a soil water content of 25%, then 14 ppm is approximately $2 \times 10^{-4}$ M. Guenzi and McCalla (1966) suggest that phenolics were likely present in higher concentrations in small localized areas. Lynch (1983) reported that acetic acid was likely to be found in the highest concentrations close to pieces of residues. He reported that concentrations as high as $1 \times 10^{-2}$ M was measured next to barley (*Hordeum vulgare* L.) straw. Gussin and Lynch (1982) indicated that only relatively small proportions of plant root systems need be exposed to acetic acid to adversely affect growth.

It is not unreasonable to assume that the concentrations

which were inhibitory to corn radicle growth would occur in the field for both volatile fatty acids and phenolic acids in small localized areas. In almost all bioassays where acidity had an effect on corn radicle growth, decreasing pH increased activity of the acid. There was also evidence that all hybrids may not be affected similarly by the presence of these acids. The combination of phenolic acids may be important since they appear to affect corn radicle growth in a non-additive manner.

Table 7.1 Mean squares and coefficients of variation from analyses of variance for bioassays of various volatile fatty acids and volatile fatty acid combinations on radicle lengths expressed as percent of control.

| Source of Variation | df | Volatile fatty acids | | | | |
|---|---|---|---|---|---|---|
| | | Acetic | Propionic | Butyric | Acetic Propionic | Acetic Butyric |
| Block (A) | 1 | 1528.8* | 1493.5** | 2662.7** | 4106.1** | 1083.8* |
| Error A | 6 | 203.4 | 70.6 | 57.3 | 108.7 | 148.5 |
| pH (B)+ | 1 | 773.9** | 921.8** | 136.1 | 71.4 | 4254.4** |
| Concentration (C)+ | 4 | 1160.4** | 16425.1** | 26910.2** | 549.2** | 1572.6** |
| Hybrid (D)+ | 2 | 144.2 | 696.1** | 387.2** | 41.4 | 664.6** |
| B X C | 4 | 123.2 | 405.2** | 242.8** | 278.2* | 307.6* |
| B X D | 2 | 130.0 | 629.7** | 406.4** | 101.7 | 66.1 |
| C X D | 8 | 124.7 | 220.1** | 130.9* | 41.5 | 86.9 |
| B X C X D | 8 | 19.0 | 90.1 | 101.6 | 146.5 | 45.6 |
| A X B | 1 | 3.1 | 1766.2** | 0.1 | 98.7 | 89.7 |
| A X C | 2 | 587.7** | 20.2 | 210.2* | 484.8* | 9.9 |
| A X D | 4 | 266.5* | 252.4** | 210.0** | 443.0** | 212.9 |
| Residual error | 196 | 87.7 | 67.8 | 57.0 | 109.2 | 109.6 |
| CV (%) | | 10.0 | 9.6 | 9.3 | 10.9 | 10.8 |

+ Significance was determined by pooling residual error with significant interaction.
* ** Significant at the 5% and 1% levels of probability, respectively.

Table 7.2 Effect of various volatile fatty acids and pH levels on corn radicle length expressed as percent of control.

| Acid | pH 4.5 | | pH 7.5 |
|---|---|---|---|
| Acetic | 91.7 b* | * | 95.3 b |
| Propionic | 83.8 c | * | 87.7 c |
| Butyric | 82.3 c | | 80.8 d |
| Acetic-Propionic | 96.8 a | | 95.8 b |
| Acetic-Butyric | 92.5 b | * | 100.9 a |

* Within row means separated with this sign are significantly different according to a LSD test at the 5% level.

\+ Within column means followed by the same letter are not significantly different at the 5% level.

Table 7.3 Effect of various volatile fatty acids on radicle length expressed as percent of control for three corn hybrids.

| Acid | Hybrid: Pioneer 3737 | Pioneer 3949 | Hyland 2260 | LSD* |
|---|---|---|---|---|
| Acetic | 92.6 b** | 92.8 b | 95.4 a | 2.9 |
| Propionic | 89.1 c | 84.5 c | 83.6 b | 2.6 |
| Butyric | 84.1 d | 80.5 d | 80.1 c | 2.3 |
| Acetic-Propionic | 96.9 a | 95.5 b | 96.4 a | 3.2 |
| Acetic-Butyric | 94.0 b | 99.7 a | 96.5 a | 3.2 |

* Least significant difference at the 5% level comparing differences among hybrids within acids.

** Within column means followed by the same letter are not significantly different at the 5% level.

Table 7.4 Effect of various volatile fatty acids and pH levels on radicle length expressed as percent of control for three corn hybrids.

| Acid | pH | Hybrid | | | LSD+ |
|---|---|---|---|---|---|
| | | Pioneer 3737 | Pioneer 3949 | Hyland 2260 | |
| Propionic | 4.5 | 86.6 | 80.1 | 84.7 | |
| | 7.5 | 91.7 | 88.9 | 82.5 | 3.6 |
| Butyric | 4.5 | 87.4 | 80.3 | 79.2 | |
| | 7.5 | 80.0 | 80.0 | 80.0 | 3.3 |

+ Least significant difference at the 5% level within each VFA bioassay.

Table 7.5 Test of non-additive effects between acetic-propionic and acetic-butyric acids.

| Acid Combination | Comparison+ | Standard error++ | t(P)+++ |
|---|---|---|---|
| Acetic-Propionic | 1.8 | 1.4 | 1.286(0.20) |
| Acetic-Butyric | 1.5 | 1.3 | 1.153(<0.20) |

+ Formula: combination(A,B) - 0.5(A + B).

++ Standard error of the comparison based upon a variance estimate of 88.2 and 84.8 for the acetic-propionic and acetic-butyric combinations, respectively, each with 196 df.

+++ Significance probability for a two-tailed student's t-test.

Table 7.6 Mean squares and coefficients of variation from analyses of variance for bioassays of various phenolic acids and phenolic acid combinations on radicle lengths expressed as percent of control.

| Source of Variation | df | Phenolic acid | | | | | |
|---|---|---|---|---|---|---|---|
| | | Vanillic | Hydroxy-benzoic | Ferulic | Coumaric | Coumaric Ferulic | Coumaric Vanillic |
| Block (A) | 1 | 1412.2** | 3968.1** | 2012.8* | 1858.4** | 606.7 | 0.1 |
| Error A | 6 | 68.4 | 160.4 | 176.0 | 70.5 | 134.9 | 214.6 |
| pH (B)+ | 1 | 1.6 | 374.9** | 0.3 | 137.5 | 35.3 | 1336.9 |
| Concentration (C)+ | 4 | 665.8** | 241.4 | 204.7 | 340.5* | 127.0 | 434.4* |
| Hybrid (D)+ | 2 | 202.9 | 146.7 | 798.0** | 1564.7** | 720.6** | 396.6 |
| B X C | 4 | 84.9 | 40.6 | 157.3 | 86.6 | 166.9 | 108.6 |
| B X D | 2 | 289.5* | 226.2 | 105.0 | 1008.7** | 340.7** | 494.9* |
| C X D | 8 | 47.9 | 37.3 | 94.5 | 168.2 | 109.7 | 194.7 |
| B X C X D | 8 | 102.1 | 92.3 | 42.4 | 192.4* | 109.3 | 63.4 |
| A X B | 1 | 557.3* | 382.9* | 1.8 | 426.0 | 541.2* | 2112.2** |
| A X C | 2 | 778.0** | 856.2** | 281.9 | 275.0 | 259.4 | 230.7 |
| A X D | 4 | 424.0** | 401.6** | 181.5 | 167.2 | 199.8 | 58.5 |
| Residual error | 196 | 83.7 | 95.2 | 99.1 | 96.5 | 106.2 | 137.7 |
| CV (%) | | 9.3 | 9.9 | 10.1 | 10.1 | 10.2 | 11.7 |

+ Significance was determined by pooling residual error with significant interaction.
* ** Significant at the 5% and 1% level of probability, respectively.

Table 7.7 Effect of various phenolic acids and pH levels on corn radicle length expressed as percent of control.

| Acid | pH 4.5 | | pH 7.5 |
|---|---|---|---|
| Vanillic | 98.4 b+ | | 98.5 a |
| Hydroxybenzoic | 97.4 b | * | 99.9 a |
| Ferulic | 98.5 b | | 98.6 a |
| Coumaric | 96.9 bc | | 98.5 a |
| Coumaric-Ferulic | 101.4 a | | 100.7 a |
| Coumaric-Vanillic | 94.8 c | * | 99.5 a |

\+ Within column means followed by the same letter are not significantly different at the 5% level.

* Within row means separated by this sign are significantly different according to a LSD test at the 5% level.

Table 7.8 Effect of various phenolic acids on radicle length expressed as percent of control for three corn hybrids.

| Acid | Hybrid: Pioneer 3737 | Pioneer 3949 | Hyland 2260 | LSD+ |
|---|---|---|---|---|
| Vanillic | 96.7 ab++ | 98.7 b | 99.9 bc | 2.8 |
| Hydroxybenzoic | 97.2 ab | 99.8 ab | 99.0 bc | 3.0 |
| Ferulic | 94.8 bc | 99.7 ab | 101.0 b | 3.4 |
| Coumaric | 93.3 c | 102.2 a | 97.6 c | 3.0 |
| Coumaric-Ferulic | 99.7 a | 98.9 ab | 104.7 a | 3.2 |
| Coumaric-Vanillic | 96.5 b | 95.3 c | 99.6 bc | 3.6 |

\+ Least significant difference at the 5% level comparing differences among hybrids within acids.

++ Within column means followed by the same letter are not significantly different at the 5% level.

Table 7.9 Effect of various phenolic acids and pH levels on radicle length expressed as percent of control for three corn hybrids.

| Acid | pH | Hybrid: Pioneer 3737 | Hybrid: Pioneer 3949 | Hybrid: Hyland 2260 | LSD+ |
|---|---|---|---|---|---|
| Vanillic | 4.5 | 96.8 | 100.4 | 97.8 | |
| | 7.5 | 96.6 | 96.9 | | 101.8.0 |
| Coumaric | 4.5 | 88.0 | 103.8 | 98.4 | |
| | 7.5 | 98.0 | 100.0 | | 96.8.3 |
| Coumaric- | 4.5 | 99.1 | 101.7 | 103.4 | |
| Ferulic | 7.5 | 100.3 | 96.1 | 105.5 | 4.5 |
| Coumaric- | 4.5 | 96.4 | 93.3 | 94.5 | |
| Vanillic | 7.5 | 96.6 | 97.2 | 104.6 | 5.1 |

+ Least significant difference at the 5% level within each phenolic bioassay.

Table 7.10 Test of non-additive effects between coumaric-ferulic and coumaric-vanillic acids.

| Acid combination | Comparison+ | Standard error++ | t(P)+++ |
|---|---|---|---|
| Coumaric-Ferulic | 9.6 | 1.4 | 6.76(>0.001) |
| Coumaric-Vanillic | -6.0 | 1.5 | -4.02(>0.001) |

+ Formula: combination(A,B) - 0.5(A + B).
++ Standard error of the comparison based upon a variance estimate of 96.3 and 105.9 for the Coumaric-Ferulic and Coumaric-Vanillic combinations, respectively, each with 196 df.
+++ Significance probability for a two-tailed student's t-test.

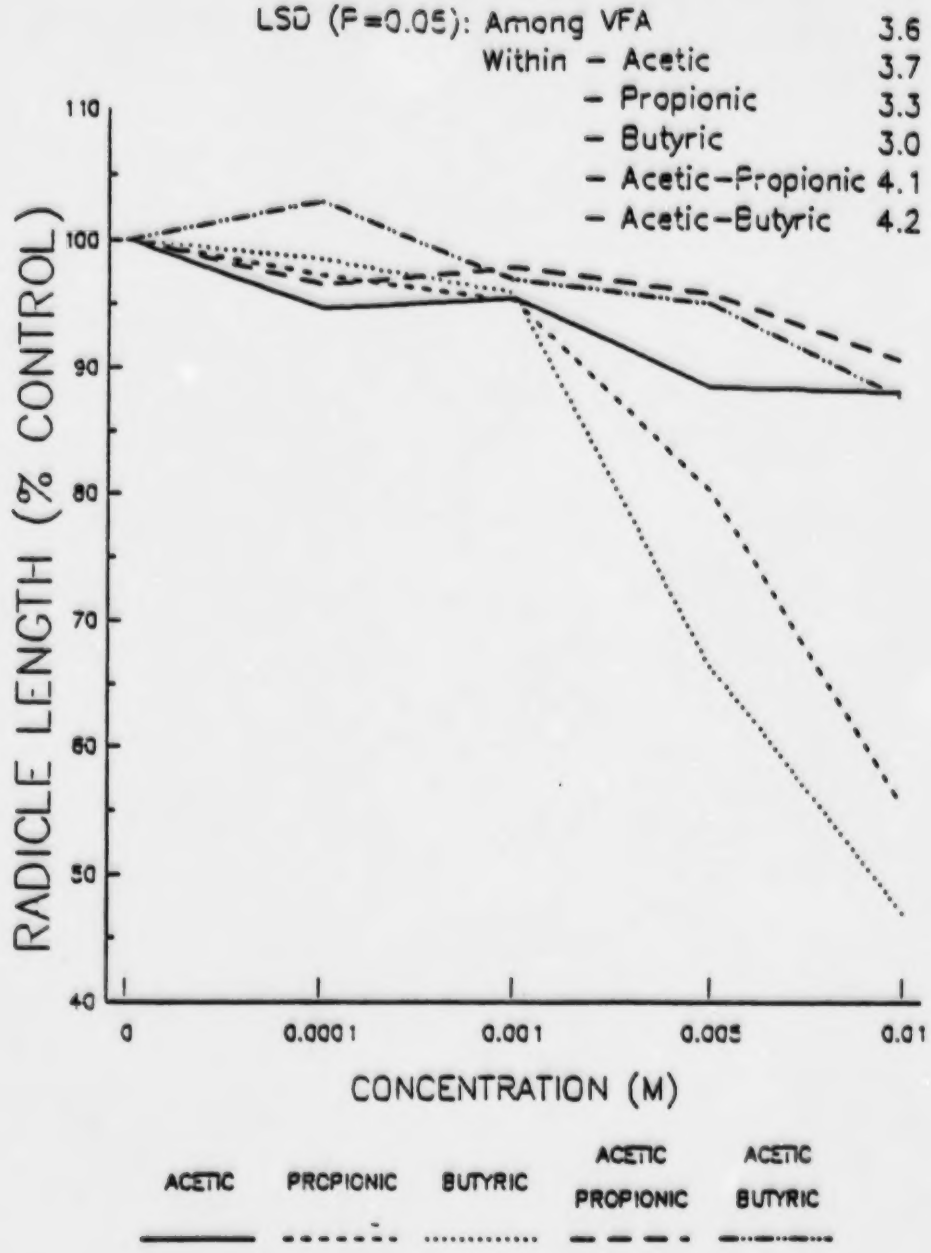


**Figure 7.1** **Effect of various concentrations of volatile fatty acids and volatile fatty acid combinations on corn radicle length (expressed as percent of control).**

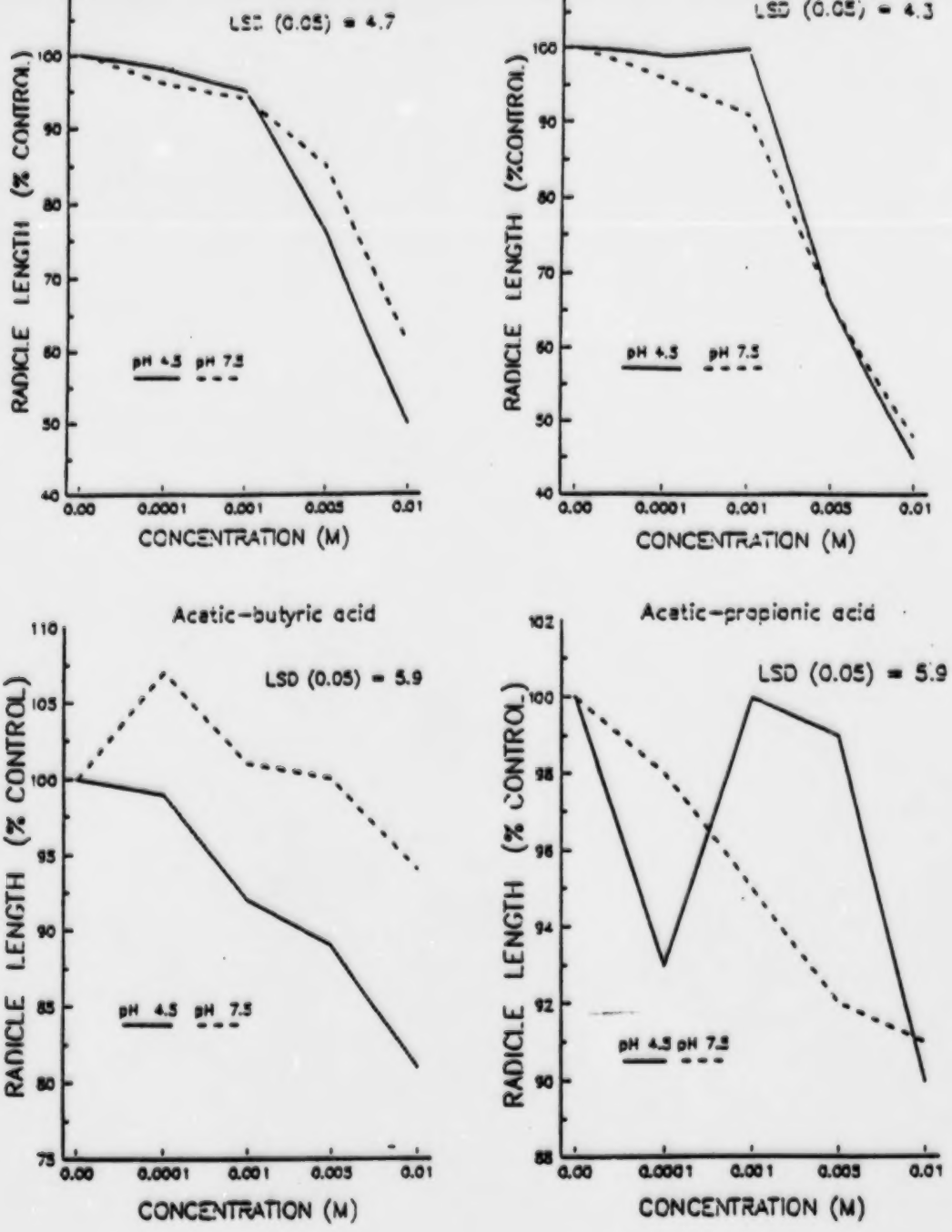


**Figure 7.2 Effect of various concentrations of volatile fatty acids adjusted to two pH levels on corn radicle lengths (expressed as percent of control).**

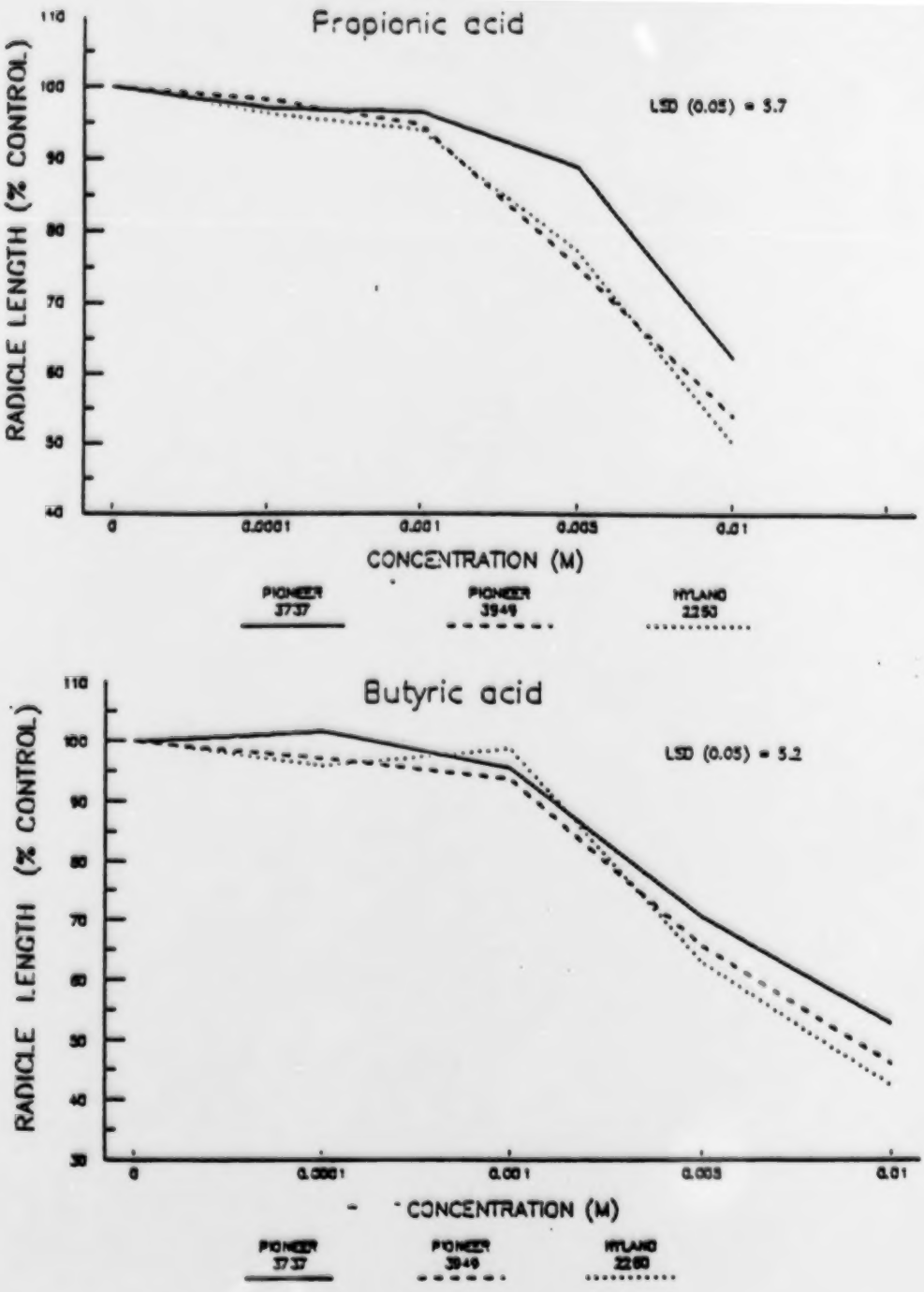


**Figure 7.3** Effect of various concentrations of volatile fatty acids on radicle lengths expressed as percent of control for three corn hybrids.

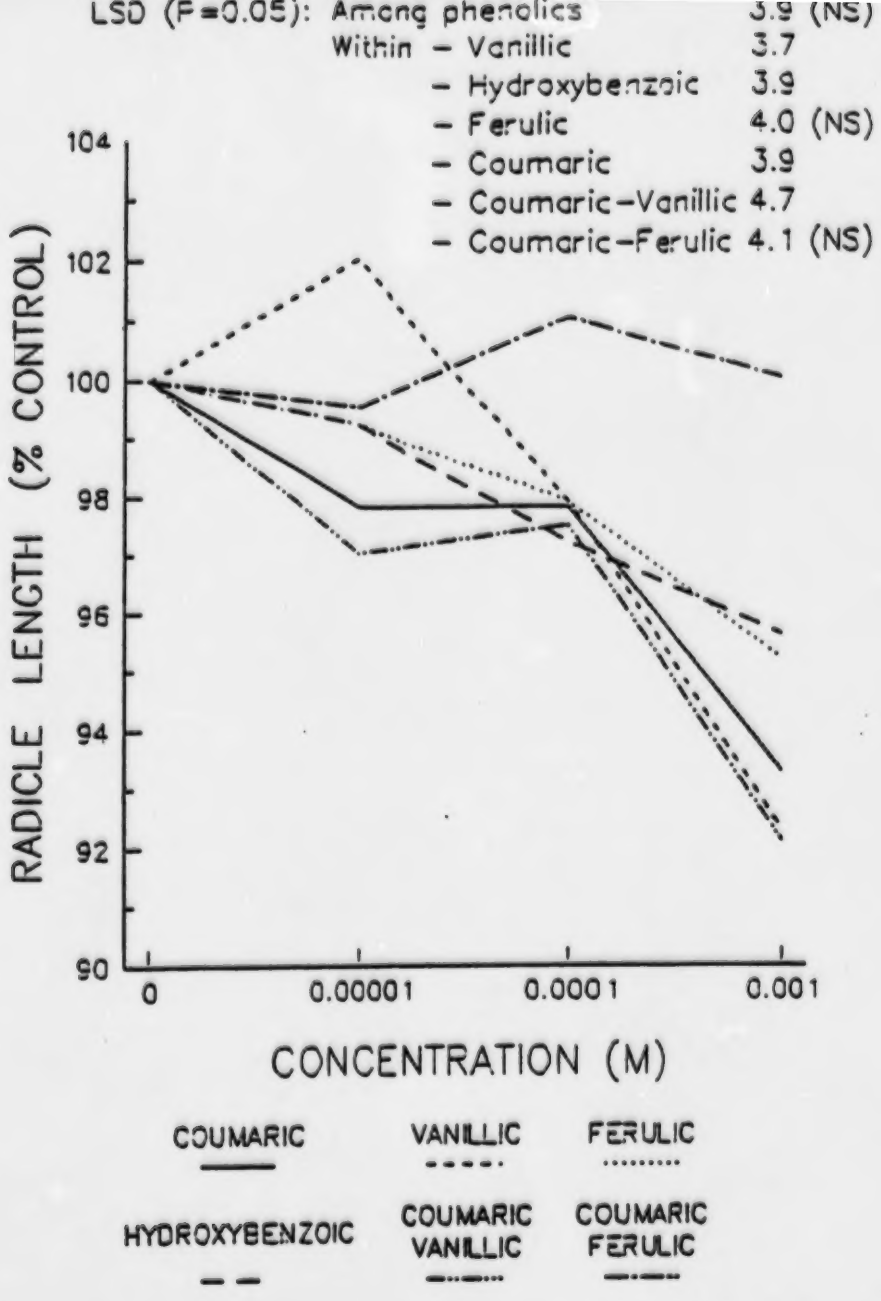


**Figure 7.4** **Effect of various concentrations of phenolic acids and phenolic acid combinations on corn radicle length (expressed as percent of control).**

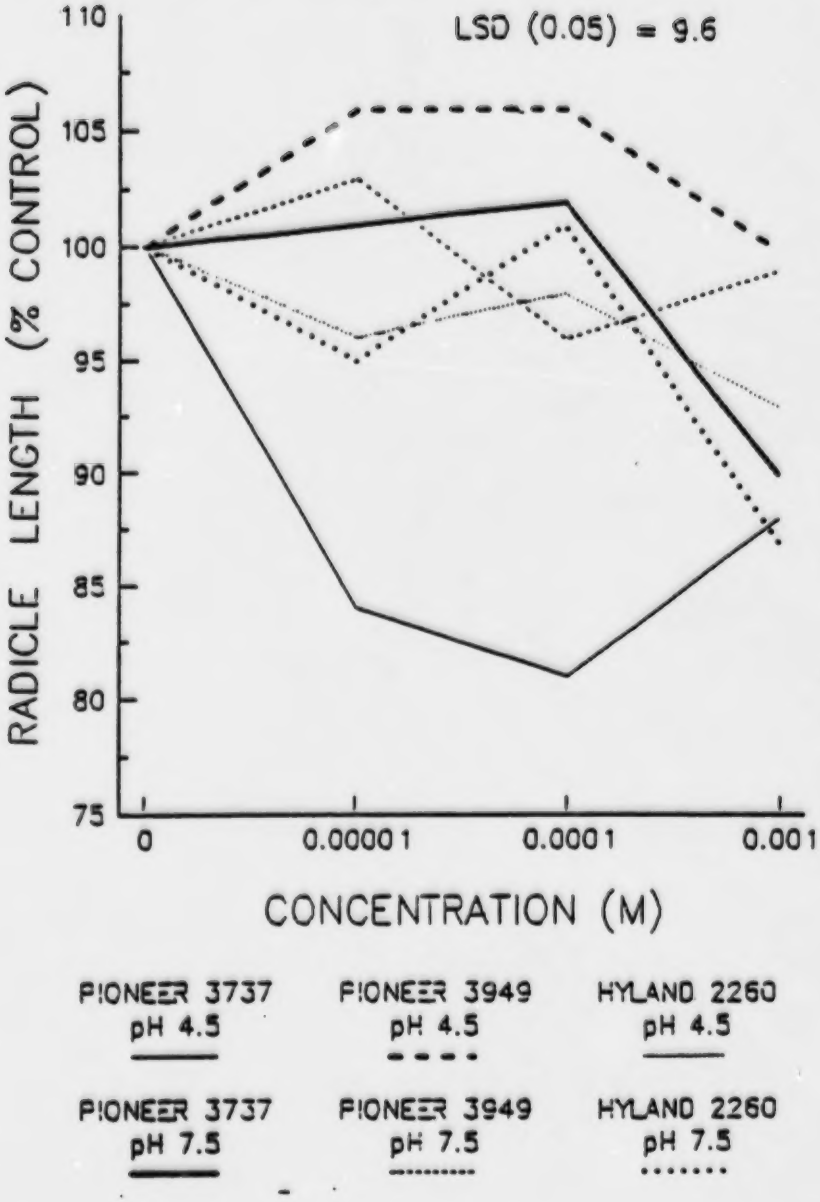


**Figure 7.5** Effect of various concentrations of coumeric acid adjusted to two pH levels on radicle lengths of three corn hybrids (expressed as percent of control).

## 8.0. CHAPTER 8 EFFECTS OF VOLATILE FATTY ACIDS AND PHENOLIC ACIDS ON WINTER WHEAT BIOASSAYS

### 8.1. OBJECTIVE

To determine if winter wheat varieties showed differences in sensitivity to individual volatile fatty acids with molar concentrations between $10^{-2}$ and $10^{-4}$ and phenolic compounds with molar concentrations between $10^{-3}$ and $10^{-4}$.

### 8.2. MATERIALS AND METHODS

The study included three varieties of winter wheat common to southwestern Ontario; Harus, Augusta and Fredrick. The volatile fatty acid (VFAs) treatments consisted of test solutions of acetic, propionic and butyric acids at concentrations of $10^{-4}$, $10^{-3}$, $10^{-4}$, $10^{-3}$ and $10^{-2}$ M. The phenolic compounds consisted of *p*-hydroxybenzoic, *p*-coumaric and vanillic acids at concentrations of $10^{-4}$, $10^{-3}$, $10^{-4}$ and $10^{-3}$ M. Five seeds were placed in a radial pattern, with the micropyle oriented towards the centre, on filter paper (Whatman #42) in a petri dish (110 x 15 mm). A second filter paper was placed over the seeds and 3 mL of test solution was pipetted into the petri dish, soaking both filter papers. The lid was placed on the dish, sealed with parafilm and incubated in the dark for 3 d at a constant temperature (25°C) and a relative humidity of 98 %. Each test solution treatment was replicated 3 times. At the end of three days, the lengths of coleoptile and radicle from each seed were recorded to the nearest half millimetre. Measurements less than 0.5 mm were recorded as

zero. The control treatment consisted of distilled, deionized water. The average of coleoptile and radicle lengths for each petri dish were determined for subsequent statistical analysis.

Three independent controls representing the three varieties were used in the bioassays. To obtain statistical comparisons of treatments among varieties required an index which measured each treatment (T) response in relation to its control (C) response (index = 100 [T/C]). Index values were subjected to frequency distribution analysis to determine normality. The coleoptile and radicle measurements with phenolic acid treatments and radicle measurements with the VFA treatments resulted in a normal distribution of the data. The frequency distribution analysis of the coleoptile measurements with VFA treatments resulted in a significant two tailed t test ($H_o$ skewness = 0, P = 0.1). Further analysis of the data showed that the untransformed index for coleoptile growth with VFA treatment was the closest to a normal distribution (Appendix B). Analysis of variance for the percent of control was carried out with Cohort Software (1986). The means were separated on the basis of least significant difference at 0.05 probability level.

**8.3. RESULTS**

The effects of the individual compounds on radicle and coleoptile growth varied with variety. Mean lengths of untreated coleoptiles with Harus, Augusta and Fredrick

varieties were 25, 24 and 33 mm, respectively. Mean lengths of untreated radicles with Harus, Augusta and Fredrick varieties were 60, 56 and 53 mm, respectively. Mean index values of radicle growth after 3 d was 94 with the VFA treatments and 109 with the phenolic acid treatments at the $10^{-4}$ molar concentration. Mean index values of coleoptile growth was 95 with the VFA treatments and 102 with the phenolic acid treatments at $10^{-4}$ M. In contrast, at $10^{-2}$ M indices for coleoptile and radicle for VFA treatments were 11 and 1 and for phenolic acids 92 and 83, respectively. Individual compounds were inhibitory or stimulatory depending on variety, concentration and appendage measured. Table 8.1 summarizes the statistical analysis of a three-way ANOVA. Almost all main effects were significant at least at P=0.05 with coleoptile length irrespective of chemical group. The exception was the main effect of phenolic acid concentration on coleoptile length. The main effects of both acid groups and concentrations were significant at P=0.05 with radicle length. Main effect of variety was nonsignificant irrespective of chemical group.

Index data are presented in Table 8.2 for the volatile fatty acid bioassay and Table 8.3 for the phenolic acid bioassay. The significant variety effect with respect to coleoptile length, irrespective of chemical group, was probably due to the inherent biological differences among the varieties. The Fredrick variety was inhibited to the greatest

extent followed by Harus and Augusta irrespective of chemical group tested. However the coleoptile response was not significantly different between Fredrick and Harus or Harus and Augusta varieties with the VFA bioassay. The Augusta coleoptile response with the phenolic acid bioassay was significantly less inhibited than the other two varieties.

The presence of acetic acid caused significant inhibition of coleoptile and radicle growth in comparison to propionic and butyric acids. All VFAs at $10^{-2}$ M caused severe inhibition to coleoptile growth and almost eradicated radicle growth (Table 8.2). Concentrations of $10^{-4}$ and $10^{-4}$ M were significantly less inhibitory to coleoptile and radicle growth in comparison to the $10^{-3}$ M concentration.

Vanillic acid was significantly more inhibitory than *p*-hydroxybenzoic and *p*-coumaric acids; the latter were actually stimulatory to coleoptile growth. However, with radicle growth, only vanillic acid was significantly more inhibitory than *p*-coumaric acid. There was no significant difference between *p*-coumaric acid and *p*-hydroxybenzoic acid with either coleoptile or radicle growth, although the *p*-coumaric treatment was significantly stimulatory. Coleoptile growth was significantly reduced by phenolic acid concentration of $10^{-3}$ M. A concentration of $10^{-3}$ M was the least inhibitory. Phenolic acid concentrations of $10^{-3}$ M significantly inhibited radicle growth.

## 8.4. DISCUSSION

As Kimber (1967) reported, radicle growth was inhibited to a greater extent than coleoptile growth by the presence of the VFAs tested. VFA toxicities were such that at high concentrations, coleoptile and radicle inhibition was almost complete. Lynch et al. (1976) reported inhibition of coleoptile and radicle growth at 78 and 68 %, respectively with a solution containing 15 mM acetic acid, 2 mM propionic acid and 1 mM butyric acid. Individual solutions of acetic, propionic and butyric acids with $10^{-3}$ M concentration caused inhibition of coleoptile growth of 65, 83 and 80 and of radicle growth of 63, 78 and 75 percent of untreated seeds, respectively. Acetic acid was the most inhibitory followed by butyric and propionic acids to both coleoptile and radicle growth.

Krogmeier and Bremner (1990) stated that VFAs derived from crop residues in soil did not occur at concentrations which would cause adverse effects on seed germination and early growth. The study reported here showed that concentrations reported in literature (10 to $15 \times 10^{-3}$ M acetic acid) caused inhibition of both coleoptiles and radicles of 35 and 37 %, respectively.

Guenzi et al. (1967) found differences among wheat varieties in response to germination and growth, especially in shoot growth when subjected to toxins. Inhibition of shoot growth ranged from 11 to 36 %. In the current study, Fredrick

showed greatest reduction in coleoptile growth (30 %) with VFAs. There was inhibition to radicle growth but no significant difference between varieties as Guenzi et al. (1967) reported with 9 wheat varieties. In the presence of phenolic acids, Fredrick was again most inhibited with respect to coleoptile (8 %) and radicle growth (3 %). A stimulatory response to the presence of phenolic acids with both coleoptile (9 %) and radicle growth (3 %) occurred with Augusta. Guenzi et al. (1967) reported no stimulatory effects on coleoptile or radicle growth with straw extracts.

The phenolic compounds included in the study are cited in the literature as candidates for causing phytotoxicity. Guenzi and McCalla (1966) concluded that *p*-coumaric acid could be released in quantities which would affect plant growth. In the current study it was found that *p*-coumaric acid as well as *p*-hydroxybenzoic acid resulted in a slightly stimulatory effect on coleoptile and radicle growth for the three wheat varieties tested. The differential response of the coleoptile and radicle growth is, in general agreement with Leather and Einhellig (1988), that the response to phytotoxins is not always linear over a range of concentrations.

## 8.5. SUMMARY

Bioassays confirmed that acetic acid was inhibitory to coleoptile and radicle extension irrespective of variety or concentration of solution. Vanillic acid was inhibitory whereas *p*-hydroxybenzoic and *p*-coumaric acids were slightly

stimulatory to both coleoptile and radicle growth.

Varietal differences in coleoptile growth were found with both chemical groups tested. Although radicle growth was inhibited there were no significant differences among varieties.

Table 8.1 Summary of a three-way ANOVA of VFA and phenolic acid of coleoptile and radicle indices with three wheat varieties

| Source | VFAs | | Phenolic Acids | |
|---|---|---|---|---|
| | coleoptile | radicle | coleoptile | radicle |
| **main effects** | | | | |
| variety | *[1] | ns | ** | ns |
| acid | *** | ** | ** | ** |
| concentration | *** | *** | ns | ** |
| **interaction effects** | | | | |
| variety x acid | ns | ns | ns | ns |
| variety x concentration | ns | ns | ns | ns |
| acid x concentration | ns | ns | ns | ns |
| variety x acid x concentration | ns | ns | ns | ns |

[1] * = = 0.05, ** = = 0.01, *** = = 0.001, ns - not significant

Table 8.2 Index value means of variety, VFA and concentration affecting coleoptile and radicle growth

| Source | | Coleoptile | Radicle |
|---|---|---|---|
| variety | | | |
| | Harus | 74.2[ab]* | 72.7[a] |
| | Augusta | 83.9[a] | 73.2[a] |
| | Fredrick | 69.5[b] | 69.7[a] |
| volatile fatty acid | | | |
| | acetic | 64.8[b] | 62.7[b] |
| | propionic | 83.2[a] | 78.4[a] |
| | butyric | 79.7[a] | 74.5[a] |
| concentration (M) | | | |
| | $10^{-4}$ | 94.7[a] | 94.3[a] |
| | $10^{-5}$ | 93.3[a] | 91.3[ab] |
| | $10^{-6}$ | 93.4[a] | 93.1[a] |
| | $10^{-7}$ | 86.6[a] | 79.5[b] |
| | $10^{-8}$ | 11.3[b] | 1.1[c] |

* significant within a column for each main effect

Table 8.3 Index value means of variety, phenolic acids and concentration affecting coleoptile and radicle growth

| Source | Coleoptile | Radicle |
|---|---|---|
| variety | | |
| Harus | 97.7[b] | 100.1[a] |
| Augusta | 108.6[a] | 103.4[a] |
| Fredrick | 91.8[b] | 96.7[a] |
| phenolic acid | | |
| vanillic | 89.7[b] | 89.9[b] |
| *p*-coumaric | 106.2[a] | 108.6[a] |
| *p*-hydroxybenzoic | 102.2[a] | 101.5[ab] |
| concentration (M) | | |
| $10^{-4}$ | 102.4[ab] | 109[a] |
| $10^{-3}$ | 104.9[a] | 105.2[a] |
| $10^{-4}$ | 98.2[ab] | 102.8[a] |
| $10^{-3}$ | 91.9[b] | 83.1[b] |

[*] significant within a column for each main effect

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# APPENDIX A SELECTED MANAGEMENT PRACTICES FOR THE PREVIOUS CROPS

Table A.1 Selected management practices for the previous crops

| Previous Crop | Variety | Seeding Rate | Herbicides | | Fertilizer | | |
|---|---|---|---|---|---|---|---|
| | | | Name | Rate | N | $P_2O_5$ | $K_2O$ |
| Corn | Pioneer 3902 | 73 000 pl $ha^{-1}$ | Dicamba<br>Metolachlor | 0.6<br>2.4 | 140 | 38 | 38 |
| Soybeans | King Grain KG 30 | 110 kg $ha^{-1}$ | Linuron<br>Metolachlor | 1.1<br>2.3 | 10 | 38 | 19 |
| Fall Rye | Danko | 170 kg $ha^{-1}$ | - | - | - | - | - |
| Wheat | Katepwa | 100 kg $ha^{-1}$ | MCPB/MCPA (15:1) | 1.4 | 70 | 38 | 19 |
| Barley | Leger | 100 kg $ha^{-1}$ | MCPB/MCPA (15:1) | 1.4 | 70 | 38 | 19 |
| Red Clover | Florex | 14 kg $ha^{-1}$ | - | - | - | - | - |
| Canola+ | Global | 20 $seed^{-1}$ | Triflurali n | 1.0 | 70 | 38 | 19 |

+ An insecticide, Carbyryl, was applied to control flea beetles at a rate of 1.0 kg $ha^{-1}$.

## APPENDIX B STATISTICAL ANALYSIS OF TRANSFORMED COLEOPTILE LENGTHS WITH VFA TREATMENTS

**Statistical analysis of transformed coleoptile lengths with VFA treatments**

Sokal and Rohlf (1969) recommended that percentage values be transformed by the arcsin of the square root of the proportion ( $\arcsin[\sqrt{T(2C^{-1})}]$ ) to normalize the data. Therefore to obtain a ratio index of treatment (T) to control (C) with values ranging between -1 and 1, required for the arcsin transformation, all treatment lengths with chemical treatments were divided by two times the respective control ($T \cdot 2C^{-1}$) treatment value (G. Kachanoski, 1991 personal communication). Frequency distribution analysis resulted in a highly significant two tailed t test of skewness ($H_0 = 0$, $P < 0.01$). The high number of values in the class width 40 to 60 probably contributed to the higher degree of skewness of transformed data. Further analysis by ANOVA of the transformed data is presented in Table B.1. The transformation resulted in similar significant main effects but also added a significant interaction effect between VFA x concentration (Table B.2). The VFA x concentration effect with coleoptile length was likely due to the much higher percent inhibition with acetic acid and higher percent stimulation with propionic acid. However, as the concentrations of propionic and butyric acids decreased the response of coleoptile growth differed. Below $10^{-4}$ M propionic acid stimulated coleoptile growth and butyric acid slightly inhibited growth.

Table B.1 Summary of a three-way ANOVA of VFA and phenolic acids on coleoptile growth data with three wheat varieties

| Source | VFAs |
|---|---|
| | coleoptile |
| **main effects** | |
| variety | *[1] |
| VFA | * |
| concentration | *** |
| **interaction** | |
| variety x acid | ns |
| variety x concentration | ns |
| VFA x concentration | * |
| variety x acid x concentration | ns |

[1] * = $\alpha$ = 0.05, ** = $\alpha$ = 0.01, *** = $\alpha$ = 0.001, ns - not significant

Table B.2 Transformed means reconverted to percent of control with 2-way interaction of volatile fatty acids by concentration

| VFA | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $10^{-6}$ | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ |
| acetic | 79.4 | 72.6 | 77.6 | 73.6 | 0.1 |
| propionic | 106.8 | 109.2 | 101.6 | 90.6 | 0.1 |
| butyric | 97.4 | 99. | 99.6 | 94.2 | <.1 |

Steel and Torrie (1980) stated that, if the range of percentages is between 30 and 70, the arcsin transformation is not required. Since most of remaining values of percent control were within the 30 to 70 percent range and frequency distribution tests found them to be normalized, the rest of the statistical analysis was done with untransformed index data.